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REVISED ADDENDUM NO. 7
QUALITY ASSURANCE PROJECT PLAN
NATURAL ATTENUATION STUDY

FOR THE

BLACKWELL LANDFILL
DUPAGE COUNTY, ILLINOIS

Montgomery Watson File No. 1252008

Prepared For:

Forest Preserve District of DuPage County
DuPage County, Illinois

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June 1998

MONTGOMERY WATSON

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APPROVALS:

U.S. EPA Region V Project Manager	Montgomery Watson QA Officer
U.S. EPA Region V Quality Assurance Reviewer	First Environmental Laboratory QA Officer
Forest Preserve District Project Manager	First Environmental Laboratory Director
Montgomery Watson Project Manager	Keystone Laboratory Director
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INTRODUCTION

A Quality Assurance Project Plan (QAPP) was previously submitted in August 1996 as part of the Pre-Design Investigation Work Plan for the Blackwell Landfill. The QAPP presented the organization, objectives, functional activities, and specific quality assurance (QA) and quality control (QC) procedures for planned sampling and analytical activities. The QAPP also described specific protocols for sampling, sample handling and storage, chain of custody, and laboratory and field analyses.

The following six addenda to the QAPP have been submitted since August 1996:

- Addendum No. 1 was prepared as part of the Expedited Final Design Report for the Leachate Collection System (LCS) (Montgomery Watson, May 1997), and provided additional details for sampling and analysis activities associated with the proposed LCS construction.

- Addendum No. 2 described additional quality assurance and quality control activities associated with construction of Cap Repairs.

- Addendum No. 3 provided an updated list of groundwater monitoring wells to be included in the quarterly groundwater monitoring program.

- Addendum No. 4 was prepared to supplement the August 1996 QAPP and an August 1990 Field Sampling Plan, which addressed sediment sampling. This addendum included updated tables that listed requirements for the analysis of soil samples collected during the North Stormwater Pipe Soil Investigation and water samples collected during surface water sampling of Sand Pond.

- Addendum No. 5 was prepared primarily to include alternative analytical methods that would achieve lower detections limits for some compounds on the groundwater analyte list. The compounds were those for which the analytical method practical quantitation limit (PQL) exceeded the promulgated maximum contaminant level (MCL). In addition, this addendum included a table that was inadvertently omitted from Addendum No. 4, described changes in the instrumentation for measurement of field parameters, and presented an updated organization chart for the Blackwell Landfill Response Action.

- Addendum No. 6 revised selected sections of the August 1996 QAPP. These revisions were requested in a March 23, 1998 letter from the U.S. EPA.

This Addendum No. 7 has been prepared to provide the additional quality assurance and quality control procedures, and analytical methods for the proposed natural attenuation study.

Information presented in the August 1996 QAPP and subsequent addenda are applicable to the proposed natural attenuation study, except as superseded by this addenda.

1.0 PROJECT DESCRIPTION

1.2 SITE BACKGROUND INFORMATION

A network of wells has been established at the Blackwell Forest Preserve site to monitor a surficial glacial outwash aquifer and a deeper bedrock aquifer. These wells have been used to monitor groundwater quality periodically over the past 15 years. Beneath and downgradient of the landfill, the two aquifers are separated by a silty clay till that is up to five feet thick.

Historic monitoring data collected since 1983 and the results for the first round of a two-year quarterly groundwater monitoring program conducted in November 1997 indicate that groundwater in the outwash aquifer has been affected by low levels of organic contaminants in an area south of the landfill. The configuration of this area is generally similar to 1992. VOCs that were detected in November 1997 in the monitoring wells within the affected area include trichloroethene (TCE), cis-1,2-dichloroethene, and 1,1-dichloroethane. In general, fewer VOC compounds and lower concentrations were reported in 1997 than in 1992. Additional compounds reported in 1992 included benzene, 1,2-dichloropropane, chloroethane, 1,1,1-trichloroethane (TCA), tetrachloroethene (commonly referred to as perchloroethene) (PCE) and vinyl chloride. Four semi-volatile organic compounds (SVOCs), phenol, pyrene, bis(2-ethylhexyl)phthalate and di-n-octylphthalate, were reported in some of the samples collected in 1992 and November 1997. Phthalates were only detected in a few samples. Since these compounds are common field and laboratory artifacts and the occurrences do not correlate with the distributions of identified site-derived contaminants, these detections likely reflect field and/or laboratory contamination. Pyrene was only reported in one sample in 1992 at a low concentration of 1 µg/L; this detection was probably not representative of groundwater quality. Phenol was detected in many of the samples, particularly those from November 1997, these detections appear to be field artifacts.

Recently, a leachate collection system (LCS) was installed at the landfill to remove leachate and reduce the potential for leachate migration. Samples of leachate from the LCS were analyzed in December 1997, January 1998, and February 1998. The analytical results show the presence of a generally different suite of VOCs when compared to downgradient groundwater. The VOCs detected in leachate include acetone, benzene, 2-butanone, chlorobenzene, 1,1-dichloroethane, cis-1,2-dichloroethene, cis-1,2-dichloropropane, ethylbenzene, methylene chloride, 4-methyl-2-pentanone, TCE, toluene, and xylenes. It is possible that other VOCs are present but were not identified because detection levels are elevated due to the leachate matrix. Only two SVOCs, 3&4-methylphenol and phenol, were detected in leachate samples.

1.2.1 Natural Attenuation Evidence

Currently available site information indicates that natural attenuation is occurring in the upper aquifer. The principal evidence for natural attenuation includes:

- The extent of the affected area in the outwash aquifer has not changed significantly since 1992, so it is not expanding.

- The number and concentrations of VOCs in the affected area of the outwash aquifer have generally decreased since 1992, suggesting that the source has lower concentrations now than earlier.

- The differences in the suites of VOCs in leachate and downgradient groundwater suggest that some organic compounds are degrading. The VOCs detected in leachate are all potentially mobile in groundwater but many are not being detected downgradient from the landfill. It appears that the non-chlorinated VOCs (acetone, 2-butanone, 4-methyl-2-pentanone, ethylbenzene, toluene, xylenes and benzene) are being attenuated rapidly because they are not presently detected downgradient of the landfill, even in the closest monitoring wells.

- Chlorinated VOCs that are migrating at low concentrations from the landfill also appear to be degrading in groundwater, but at a slower rate than the non-chlorinated compounds. Degradation is indicated not only by generally lower concentrations in 1997 compared to 1992 and the apparently non-expanding configuration of the affected area during this five-year period, but also by the types of compounds identified. Most of the VOCs that have been detected in downgradient groundwater are typical degradation products of PCE, TCE and/or TCA solvents. For example, PCE and/or TCE commonly degrade via a sequence that may include 1,2-dichloroethene, vinyl chloride, and/or chloroethane. The dominance of these typical degradation products as downgradient groundwater contaminants is strong evidence that natural attenuation processes are occurring.

The available site data provide evidence that natural attenuation is occurring in the outwash aquifer downgradient of the landfill. Moreover, the natural attenuation processes should prevent migration of contaminants to potential receptors at levels of concern. The following plan outlines a systematic program of sampling to provide additional supporting evidence of natural attenuation at the Blackwell Site.

1.3 NATURAL ATTENUATION OBJECTIVES AND SCOPE

The study will include analyses of groundwater samples for additional natural attenuation parameters, the collection and analysis of soil samples from the outwash aquifer, and predictive modeling for the outwash aquifer. The sampling and analysis programs for groundwater and soil, and the planned scope of predictive modeling activities are described in the following sections.

1.3.1 Groundwater

During the next routine quarterly sampling event, additional groundwater samples will be collected from seven of the existing outwash aquifer wells (i.e., G-130, G-118S, G-129, G-128S, G-127, G-107S, and G-122), and from five of the existing deep aquifer wells (i.e., G-132D, G-134, G-138, G-139, and G-140D).

The groundwater samples collected from these wells will be analyzed for additional parameters to support the natural attenuation study. The additional analytical parameters are summarized below.

Natural Attenuation Parameters in Groundwater	
Total Organic Carbon (TOC)	
Biological Oxygen Demand (BOD)	
Nitrate-N	
Nitrite-N	
Sulfide	
Methane	
Ethane	
Ethene	
Alkalinity	Field Parameter
Ferrous iron (Iron II)	Field Parameter

Three laboratories will be utilized to perform these analysis. Table 1-1 provides a summary of which laboratories will perform each particular analysis by matrix. The selected laboratories are detailed below.

- First Environmental Laboratories, Inc.
  1600 Shore Road
  Naperville, Illinois 60563
  Phone: 630 778 1200
  Fax: 630 778 1233
  Contact: Bill Mottashed

- Teklab, Inc.
  5445 Horseshoe Lake Road
  Collinsville, Illinois 62234
  Phone: 618 344 1004
  Fax: 601 344 1005
  Contacts: Mike Austin/Tony Lynn

- Keystone Laboratories, Inc.
  600 East 17th Street South, Suite B
  Newtown, Iowa 50208
  Phone: 800 858 5227
  Fax: 515 792 7989
  Contact: Cathy Gilbreaith

The parameters alkalinity and ferrous iron (Iron II) will be measured in the field using HACH™ field test kits. Manufacturers' instructions for use of these kits are included in Addendum No. 4 to the Field Sampling Plan.

1.3.2 Soil

Soil samples will be collected from within the outwash aquifer at three locations: within the affected area, at the downgradient edge of the affected area, and downgradient of the affected area. Specific sampling locations will be selected in the field based on the inferred position of the leading edge of the affected area and accessibility. At each of the sampling locations, soil from approximately the same depth as the monitoring well screens in the outwash aquifer will be collected.

Chemical, physical and microbiological analyses will be performed on the soil samples submitted to the off-site laboratory. The chemical parameters are summarized below.

[illegible]
Total Organic Carbon (TOC)
Nitrate-N
Sulfate
Soil pH
Total Aerobic Heterotrophs
Aerobic Hydrocarbon Degraders
Acridine Orange Counts
Total solids

Samples will be submitted to two laboratories for analysis. Table 1-1 provides a summary of which laboratories will perform each particular analysis by matrix. The selected laboratories are shown below.

- Keystone Laboratories, Inc
  600 East 17th Street South, Suite B
  Newtown, Iowa 50208
  Phone: 800 858 5227
  Fax: 515 792 7989
  Contact: Cathy Gilbreaith

- Microbe Inotech Laboratories, Inc. (MiL, Inc.)
  12133 Bridgetown Square Dr.
  St. Louis, Missouri 63044-2616
  Phone: 800 688 9144
  Fax: 314 344 3031
  Contact: Jay Garcia

Collection of soil for analysis of these parameters will not require any specific procedures not already addressed in the Field Sampling Plan.

1.5 PARAMETERS TO BE TESTED AND FREQUENCY

Table 1-2 provides a sample network summary, by matrix, of all samples planned for collection and which analyses will be performed on each sample. This table also provides a summary of the types and number of quality control/quality assurance (QA/QC) samples to be collected. Table 1-3 provides a summary of sample volume, bottle, preservation, and packaging requirements for the parameters not previously defined in the August 1996 QAPP or subsequent addenda.

Diagrams and site maps with sample locations may be found in the natural attenuation work plan.

1.6 DATA QUALITY OBJECTIVES

Addendum 6 contains the most current data quality objectives (DQOs) summary which was based on the U.S. EPA's seven step process described in the EPAQA/G-4 (September 1994) document.

1.7 PROJECT SCHEDULE

Collection of samples for the natural attenuation study is planned to occur during the next round of routine quarterly groundwater sampling which is scheduled for late June or early July 1998. Following receipt of laboratory results, data analysis and modeling will require approximately one month to complete. On the basis of this projected schedule, a report is expected to be submitted to U.S. EPA during August 1998.

2.0 PROJECT ORGANIZATION AND RESPONSIBILITY

A revised project organization chart is provided in Figure 1. Project responsibilities remain the same as those stated in the 1996 QAPP. The specialized responsibilities of laboratory analysis have been defined by the groups listed in Section 1.3 of this addendum.

3.0 QUALITY ASSURANCE OBJECTIVES FOR MEASUREMENT DATA

No changes to this section.

4.0 SAMPLING PROCEDURES

Sampling procedures for groundwater and soil samples are described Addendum No. 4 to the Field Sampling Plan.

5.0 SAMPLE CUSTODY

No changes to this section.

6.0 CALIBRATION PROCEDURES AND FREQUENCY

Alkalinity and ferrous iron (Iron II) will be measured in the field using HACH™ field test kits. Manufacturers' instructions for use of the test kits are included in Addendum No. 4 to the Field Sampling Plan.

7.0 ANALYTICAL PROCEDURES

Groundwater and leachate samples will be analyzed by First Environmental Laboratories, Inc. with the exception of TOC which will be analyzed by Teklab, Inc. and methane, ethane, and ethene which will be analyzed by Keystone Laboratories, Inc. Soil samples will be analyzed by Keystone Laboratories, Inc. with the exception of the acridine orange counts that will be performed by Microbe Inotech Laboratory, Inc. Refer is Table 1-1 for a summary of matrices, parameters, method references, and laboratory assignments.

7.1 FIELD SCREENING ANALYTICAL PROTOCOLS

No changes to this section.

7.2 LABORATORY ANALYSIS

The SOPs for the additional natural attenuation parameters in groundwater are provided in Attachment A to this addendum. Similarly, SOPs for the additional natural attenuation parameters in soil are included in Attachment B.

8.0 INTERNAL QUALITY CONTROL CHECKS

No changes to this section.

9.0 DATA REDUCTION, VALIDATION, AND REPORTING

No changes to this section.

10.0 PERFORMANCE AND SYSTEM AUDITS

No changes to this section.

11.0 PREVENTATIVE MAINTENANCE PROCEDURES

No changes to this section.

12.0 SPECIFIC ROUTINE PROCEDURES TO ASSESS DATA PRECISION, ACCURACY, AND COMPLETENESS

No changes to this section.

13.0 CORRECTIVE ACTION

No changes to this section.

14.0 QUALITY ASSURANCE REPORTS TO MANAGEMENT

No changes to this section.

MP/ME/WB/dlp/emp
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TABLES

TABLE 1-1

Summary of Matrices, Analysis Parameters, Reporting Limits, and Analytical Laboratory Responsibilities
Natural Attenuation Study Blackwell Landfill NPL Site
DuPage County, Illinois

Analysis	Method*	Compounds	Reporting Limits	Laboratory	Data Use
GROUNDWATER					
TOC	9060	TOC	1 mg/l	Teklab, Inc.	Attenuation
BOD	SMWW 405.1	BOD	1 mg/l	First Environmental Labs	Attenuation
Nitrate (total) as N	353.3 Mod	Nitrate as N	0.05 mg/l	First Environmental Labs	Attenuation
Nitrite	354.1	Nitrite	0.005 mg/l	First Environmental Labs	Attenuation
Sulfide	376.2	Sulfide	0.05 mg/l	First Environmental Labs	Attenuation
Methane	ASTM D-1945/D-3588	Methane	0.02 mg/l	Keystone Labs	Attenuation
Ethane	ASTM D-1945/D-3588	Ethane	0.03 mg/l	Keystone Labs	Attenuation
Ethene	ASTM D-1945/D-3588	Ethene	0.03 mg/l	Keystone Labs	Attenuation
SOILS					
Total Solids	160.3	Solids	0.1%	Keystone Labs	Attenuation
TOC	9060	TOC	100 mg/kg	Keystone Labs	Attenuation
Nitrate as N	SMWW 4500-D	Nitrate	5 mg/kg	Keystone Labs	Attenuation
Sulfate	6010	Sulfur as Sulfate	100 mg/kg	Keystone Labs	Attenuation
pH	9045	pH	NA	Keystone Labs	Attenuation
Total Heterotrophs	SMWW 9215B	Total Heterotrophs	200 CFU	Keystone Labs	Attenuation
Aerobic HC Degraders	SMWW 9215B	Aerobic HC Degraders	200 CFU	Keystone Labs	Attenuation
Acridine Orange Counts	SMWW 9216 B	Direct total bacteria cell counts	100 cells/ml	MiL, Inc.	Attenuation

* - unless otherwise noted method reference is from USEPA
QAPP - Quality Assurance Project Plan
TOC - Total Organic Carbon
BOD - Biological Oxygen Demand
N - Nitrogen
Mod - Modified
mg/l - milligrams per liter
mg/kg - milligrams per kilogram
ASTM - American Standards Testing Method
SMWW - Standard Methods for the analysis of Water and Wastewater
NA - Not applicable
HC - Hydrocarbon
CFU - Colony Forming Units
MiL, Inc. - Microbe Inotech Laboratory, Inc.
COD - Chemical Oxygen Demand
TDS - Total Dissolved Solids
TSS - Total Suspended Solids
OC - Organochlorine
Attenuation - refers to parameters to assess natural attenuation

TABLE 1-2

Sample Types and Estimated Sample Quantities
Natural Attenuation Study Blackwell Landfill NPLSite
DuPage County, Illinois

Matrix	Laboratory	No. of Samples	Field Duplicates	Field Blank	MS/MSD	Total No. of Samples	Laboratory Parameters	Field Parameters
Groundwater								
	Teklab	12	2	0	0	14	TOC	Alkalinity, and ferrous iron (Iron II)
	First	12	2	2	0	16	BOD	
	First	12	2	2	1	17	Nitrate as N	
	First	12	2	2	1	17	Nitrite	
	First	12	2	2	1	17	Sulfide	
	Keystone	12	2	2	1	17	Methane	
	Keystone	12	2	2	1	17	Ethane	
	Keystone	12	2	2	1	17	Ethene	
Soil								
	Keystone	3	1	0	0	4	Solids	
	Keystone	3	1	0	0	4	TOC	
	Keystone	3	1	0	1	5	Nitrate as N	
	Keystone	3	1	0	1	5	Sulfate	
	Keystone	3	1	0	0	4	pH	
	Keystone	3	1	0	0	4	T. Heterotrophs	
	Keystone	3	1	0	0	4	HC Degraders	
	MiL, Inc.	3	1	0	0	4	Acridine Orange counts	

TABLE 1-2

Sample Types and Estimated Sample Quantities
Natural Attenuation Study Blackwell Landfill NPLSite
DuPage County, Illinois

Matrix	Laboratory	No. of Samples	Field Duplicates	Field Blank	MS/MSD	Total No. of Samples	Laboratory Parameters	Field Parameters

Notes:
- Unless otherwise noted, samples will be considered low concentration, and will be packaged and shipped accordingly.
- Full names and addresses of laboratories may be found in Section 1.3 of this addendum.
  - First - First Environmental Laboratory, Inc.
  - Teklab - Teklab, Inc.
  - Keystone - Keystone Laboratory, Inc.
  - MiL - Microbe Inotech Laboratory, Inc.
- Refer to Table 7-1 for method references. Refer to Table 1-2 of 1996 QAPP and Table 1-3 of this addendum for sample volume and preservative requirements.
- TDS aliquot to be field filtered.

No. - Number
MS/MSD - Matrix Spike/ Matrix Spike Duplicate
TOC - Total Organic Carbon
BOD - Biological Oxygen Demand
N - Nitrogen
T. - Total
HC - Hydrocarbon
COD - Chemical Oxygen Demand
TDS - Total Dissolved Solids
TSS - Total Suspended Solids
OC Pest - Organochlorine Pesticides

TABLE 1-3

Sample Quantities, Containers, Preservatives, and Packaging Requirements
Natural Attenuation Study Blackwell Landfill NPL Site
DuPage County, Illinois

Matrix	Analysis	Bottles/Jars	Preservation	Holding Time	Volume	Shipping	Packaging
Note: this table only includes matrices and analyses not included in the 1996 QAPP Table 1-2.							
Groundwater							
	TOC	125 ml HDPE	Cool to 4 C, HCl or H2SO4 to pH<2	28 days	Fill to shoulder of bottle	Shipped daily by overnight carrier	Vermiculite
	BOD	2 L HDPE	Cool to 4 C, H2SO4 to pH<2	48 hours	Fill to shoulder of bottle	Shipped daily by overnight carrier	Vermiculite
	Nitrate as N	250 ml HPDE	Cool to 4 C, H2SO4 to pH<2	28 days	Fill to shoulder of bottle	Shipped daily by overnight carrier	Vermiculite
	Nitrite	125 ml HDPE	Cool to 4 C	48 hours	Fill to shoulder of bottle	Shipped daily by overnight carrier	Vermiculite
	Sulfide	1 L HPDE	Cool to 4 C, 4 ml ZnAc plus NaOH to pH> 9	7 days	Fill to shoulder of bottle	Shipped daily by overnight carrier	Vermiculite
	Methane, ethane, and ethene	3-40 VOA vials	Cool to 4 C, HCl to pH<2	7 days	Fill completely, no headspace	Shipped daily by overnight carrier	Vermiculite
Soil							
	Total Solids	One 4-oz glass jar	Cool to 4 C	7 days	Full	Shipped daily by overnight carrier	Vermiculite
	TOC	One 4-oz glass jar	Cool to 4 C	28 days	Full	Shipped daily by overnight carrier	Vermiculite
	Nitrate as N	One 4-oz glass jar	Cool to 4 C	28 days	Full	Shipped daily by overnight carrier	Vermiculite
	Sulfate	One 4-oz glass jar	Cool to 4 C	28 days	Full	Shipped daily by overnight carrier	Vermiculite
	Total Heterotrophs	One 8-oz glass jar	Cool to 4 C	24 hours	Full	Shipped daily by overnight carrier	Vermiculite

TABLE 1-3

Sample Quantities, Containers, Preservatives, and Packaging Requirements
Natural Attenuation Study Blackwell Landfill NPL Site
DuPage County, Illinois

Matrix	Analysis	Bottles/Jars	Preservation	Holding Time	Volume	Shipping	Packaging
	Aerobic HC Degraders	One 8-oz glass jar	Cool to 4 C	5 days	Full	Shipped daily by overnight carrier	Vermiculite
	Acridine Orange Counts	One 8-oz glass jar	Cool to 4 C	24 hours	Full	Shipped daily by overnight carrier	Vermiculite
	Soil pH	One 8-oz glass jar	Cool to 4 C	24 hours	Full	Shipped daily by overnight carrier	Vermiculite

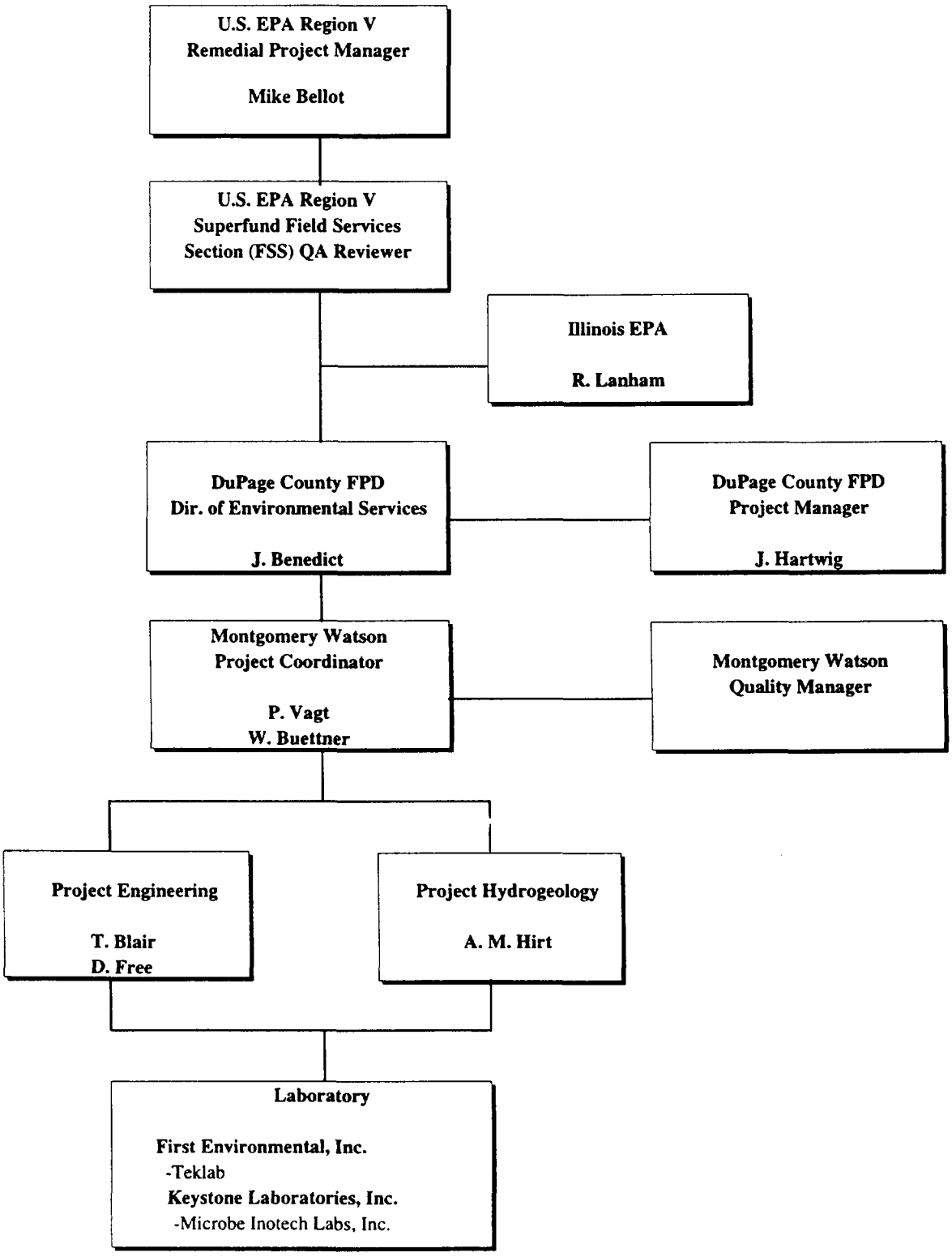
Notes:
- Holding time begins at the time the sample is collected.
- The packaging material should completely cushion the sample bottles - bottom, sides and top.

TOC - Total Organic Carbon
ml - milliliter
HDPE - High density polyethylene
C - Celsius
HCl - Hydrochloric acid
H2SO4 - Sulfuric acid
BOD - Biological Oxygen Demand
L - Liter
N - Nitrogen
ZnAc - Zinc Acetate
NaOH- Sodium Hydroxide
COD - Chemical Oxygen Demand
TDS - Total Dissolved Solids
TSS - Total Suspended Solids
OC - Organochlorine
oz - ounce
HC -Hydrocarbon

FIGURES

FIGURE 1

ORGANIZATION CHART
BLACKWELL LANDFILL RESPONSE ACTION



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ATTACHMENT A

LABORATORY SOPs FOR ADDITIONAL GROUNDWATER ANALYSES

TOTAL ORGANIC CARBON

Rev. 5/98

TEKLAB INC.
STANDARD OPERATING PROCEDURE
TOTAL ORGANIC CARBON

SCOPE

This method is applicable to drinking, surface, ground and wastewater, as well as, soils, sludge and solids. This procedure is to be used to determine the concentration of organic carbon in ground, surface and wastewaters. This method is applicable to measurement of organic carbon above 1 and less than 200 mg/l (Note: Samples with concentrations >200 mg/l must be diluted to less than 200 mg/l). Reference SW-846, EPA 600 415.2, S.M. 5310C

SUMMARY

Organic carbon is measured using a carbonaceous analyzer. this method uses an ultraviolet light and persulfate oxidation reaction to convert carbon, of all forms, to carbon dioxide. The CO_2 formed is then measured. The amount of CO_2 in a sample is directly proportional to the concentration of carbonaceous material in the sample. Carbonate and bicarbonate are inorganic forms of carbon, which must be separated from the total organic carbon. This is accomplished by degassing prior to analysis.

Soils, sludge and solids are burned in a furnace in a oxygen stream to convert organic carbon to CO_2. The analyzer uses and infrared (IR) detector to analyze the gas stream for CO_2 after the oxidation step (in either case).

DEFINITIONS

See Appendix A

INTERFERENCES

Carbonate and bicarbonate will interfere and must be removed by acidification and degassing with oxygen. However, this will reault in the loss of volatile organic compounds. Large organic particles or large, complex organic molecules may be oxidized slowly because persulfate oxidation is rate-limited. Persulfate oxidation is slow in samples containing significant concentrations of chloride by the preferential oxidation of chloride; at a concentration of 0.1% chloride, oxidation of organic matter may be inhibited completely. To remove the interference add mercuric nitrate to the persulfate solution. Samples containing large suspended solids can plug the analyzers sample lines.

SAFETY

Use caution when handling acids used in sample treatment. If samples are basic, care must be taken during acidification of the sample. Refer to the appropriate MSDSs for more safety information.

APPARATUS

1. Dohrmann Model DC80 TOC analyzer equipped with an ASM-1 autosampler for water samples and a sludge/sediment combustion attachment for soils, sludge and solids.
2. Syringe 1000 microliter for manual injection.
3. Syringe 40 microliter for sludge/solids.
4. Test tubes 25 ml for autosampler.
5. Analytical balance capable of measuring 0.1 mg.

REAGENTS

1. Potassium Hydrogen Phthalate (KHP) 2000 ppm stock solution. Dry KHP in an oven at 105 deg C for 4 hours. Weigh out 4.250 g and transfer to a 1000 ml volumetric flask. Add approximately 80 ml of deionized water. Add 1 ml of concentrated H3PO4 and dilute to volume. 2,000 micrograms = 1 ml
2. Potassium Persulfate Solution: weigh out 40 g Potassium Persulfate ($K_2S_2O_8$) and transfer to a 2000 ml volumetric flask. Add about 1,900 ml DI water and add 2 ml of concentrated H_3PO_4. Dilute to volume.
3. 10 ppm TOC standard: Dilute 5 ml of 2000 ppm stock solution to 1000 ml (in a volumetric flask) with DI water. 10 micrograms = 1 ml
4. 2 ppm TOC standard: Dilute 1 ml of 2000 ppm stock solution to 1000 ml (in a volumetric flask) with DI water. 2 micrograms = 1 ml
5. 100 ppm TOC standard: Dilute 25 ml of 2000 ppm stock solution to 500 ml (in a volumetric flask) with DI water. 100 micrograms = 1 ml
6. 500 ppm TOC standard: Dilute 25 ml of 2000 ppm stock solution to 100 ml (in a volumetric flask) with DI water. 500 micrograms = 1 ml.
7. Deionized water (DI)

SAMPLE COLLECTION, PRESERVATION AND HOLDING TIMES

See Appendix B

QUALITY ASSURANCE/QUALITY CONTROL

Maintenance: The TOC analyzer will require maint. which will include: cleaning reaction module, changing permeation dryer, installing new pump tubes, etc. Refer to the manual for the procedures All maint. must be recorded in a log.

A blank shall be run at the beginning and end of each run of analysis and every 20th sample in between. Two 10.0 mg/l standards or two QC samples, of known concentration, must be analyzed with every run of analysis. The QC samples or standards will be run at the beginning and end of every analysis (or every 20th sample; whichever is more frequent). The check standard must be between 9.5 mg/l and 10.5 mg/l for the run to be acceptable. The QC sample must be within the

range specified by the manufacturer for the run to be acceptable.

A 25 mg/l matrix spike (MS) and a matrix spike duplicate (MSD) must be analyzed with, at least every 10th sample. The matrix spike recovery (%MSR) must be between 85% and 115% for the run to be acceptable. The Relative Percent Difference (%RPD) of duplicates must be between -10% and +10% for the analysis to be acceptable.

$$1st\ \%MSR = \frac{X_2 - X_1}{10\ mg/l} * 100$$

$$2nd\ \%MSR = \frac{X_3 - X_1}{10\ mg/l} * 100$$

$$\%RPD = \frac{(X_2 - X_3)}{(X_2 + X_3)/2} * 100$$

Where,

X_1 = Concentration of unspiked sample, mg/l
X_2 = Concentration of 1st spiked sample, mg/l
X_3 = Concentration of 2nd spiked sample, mg/l

SAMPLE PREPARATION

Samples can be stored in either plastic or glass containers. Samples must be preserved with HCl or H_2SO_4 to a pH of <2. Samples should be kept cool (4 deg C) and protected from sunlight and atmospheric oxygen.

PROCEDURE

1. Make sure the short tube is in place between connection 4 and 5 on the pump side of the reaction module.
2. Turn on power to ASM-1.
3. Turn on power to analyzer.
4. Set analyzer to TOC setting and 1 ml setting for sample size.
5. Turn on pump. Place pump pickup tube in bottle of potassium persulfate solution.
6. Turn on lamp.
7. Open valve on oxygen cylinder. Make sure there is sufficient gas in the tank and the regulator is set at 35 psi.
8. With either analyzer set on the detector mode, allow the instrument to run until a steady baseline is obtained. This should take between 15 and 20 minutes. The baseline detector response should be approximately 0.0100. If it is not within +/- 0.0020, the infrared detector may need the zero adjusted. If it is determined it is not contamination in the system, refer to the detector manual for detailed directions.

9. If the instrument was calibrated recently and the calibration is not erased (the calibrate light is on) then you may check to see if the calibration is alright to use. First run a blank to flush out the system; run a 10 ppm standard. If the standard is within 5% (9.5-10.5) then the current calibration is usable. If not the instrument must be recalibrated.
10. RECALIBRATION: Push the calibrate button and hold until the light goes out. The printout on the tape should read 'NO CALIBRATION -10 PPM). Note: If samples are to be run with the ASM-1 autosampler, standardization must be carried out using the autosampler. Only when samples are to be injected directly should direct injection be used to calibrate the instrument.

    a. Using the ASM-1 autosampler: Fill 5 test-tubes with 10 ppm TOC standard and place in carousel of auto sampler. Turn carousel to position the first tube under the sample pickup tube. NOTE: The carousel rotates counter clockwise. Any attempt to turn it clockwise will cause damage to the autosampler.
    b. Push the green GO button on the manual operation side of the ASM-1.
    c. When the sample is processed it will print the result on the tape printout. When using a 10 ppm standard it must be 7.5 +/- 2.85. If it is not in this range the source of the error must be found before continuing.
    d. If the first reading is 7.50 +/- 2.85, then repeat the process with the 4 remaining tubes.
    e. After processing the last standard, push and hold the calibration button until it lights. The printout should indicate the instrument is now calibrated.

SAMPLE ANALYSIS - WATERS

1. A series of test tubes should be numbered and placed, in order, in the autosampler carousel. Place them so as the carousel turns (counterclockwise) the samples will go through in ascending order
2. Load the carousel so the first tube is a 10 ppm, 2nd is a 10 ppm, the 3rd is a 2 ppm, 4th is a blank, 5th is a 100 ppm standard and then begin loading samples.
3. Once the carousel is loaded, place it so the pickup is over the first tube. Push the auto button and the instrument will run unattended.
4. If the analyzer is to be left unattended longer than the sample run time, place the small black magnet on the carousel 2 or 3 spots behind the last tube (with the arrow facing the direction of operation)

STANDARDIZATION USING THE SLUDGE/SEDIMENT SYSTEM

1. Remove the short transfer line between ports 4 and 5 on the side of the TOC reaction module.
2. Insert the small tube coming form the combustion tube into port number 4.
3. Insert the large tubing, leading into the sample introduction tube into port 5.
4. Turn on the oxygen. Be sure there is sufficient oxygen in the tank and the regulator is set at 35 psi.
5. Turn on the furnace by pushing the button.

6. Set to the TOC setting and sample to 40 microliter.
7. Push calibrate button (if it is lighted) to erase the previous calibration.
8. Allow the furnace to warm until it has an orange glow (this may require 25 - 30 min).
9. Open sample introduction door and use tweezers to remove the small platinum sample boat. Be careful not to bend the boat holder. Close the hatch door until ready to add new sample.
10. Place a small amount of quartz wool in the boat. Replace boat in holder, through the hatch. It is important that the door is closed tightly and seals properly. Use care to insure this.
11. Slowly push the boat into the furnace and allow it to bake to remove any carbon contamination. Watch the detector response, it should increase to a maximum, then begin to return to the baseline. When it begins to come down slide the boat out of the furnace into the sample hatch. Allow to cool. The green light on the analyzer should be light.
12. When the baseline has returned, slide sample boat under the small, plugged injection port just in front of the sample hatch. Remove the plug and slowly inject 40 microliter of a 2000 ppm standard into the quartz wool boat. Replace the plug.
13. Slide the boat into the furnace and push the green start button on analyzer. Make sure the green ready light is on prior to pushing the boat into the oven.
14. When the sample is done the green ready light will come on. Slide boat out of furnace into hatch to cool. The ppm reading should be 1500 +/- 375.
15. When the sample boat is cool (4-5 min) repeat steps 12-14 three more times.
16. After four standards are done push and hold calibrate button until the light comes on.
17. Now repeat steps 12-14. when analysis is completed a reading of 2000 +/- 10 % should result.

SAMPLE ANALYSIS - SOLIDS

1. Using steps 12-14 above, analyze a blank (40 microliter DI), a 500 ppm TOC standard and a 2000 ppm standard. Results should be +/- 10%.
2. Most samples, unless they are clean water, must be weighed into sample boat. A sample weight of 2 to 40 mg may be used. If less than 100 ppm TOC is detected on the analyzer, a larger sample must be rerun.
3. Open hatch and remove boat using tweezers (and close the door) At no time touch the boat, because fingerprints will cause contamination. Place boat on an analytical balance (0.0000g) and tare. Using a spatula on soils or a disposable pipet on liquids, weigh approx. 2.0 mg (0.0020g) sample into boat.
4. Place boat back into holder and close hatch door.
5. When a ready signal is indicated by the light on analyzer (light should be on before removing boat), slide sample into furnace and press start button on analyzer. result from analyzer will print when finished.
6. If more than 2.0 mg sample is used and the concentration is >3000, the sample must be rerun using a smaller sample. If the conc is >3000 using 2.0 mg sample, the result is reported as greater than 60,000 mg/kg.
7. If the concentration on the analyzer is less than 100 ppm and 40 mg of sample was used the results are reported as less than 100 mg/kg.

CALCULATIONS

Waters conc. X dilution factors = TOC mg/l
Sludge/soils/sediments conc. X 40 / mg sample = TC mg/kg

POLLUTION PREVENTION

See Appendix D

WASTE MINIMIZATION

See Appendix D

REFERENCES:

See Appendix D

Laboratory Director/QA Officer

5/20/98
Date

Appendix A - Definitions

For the purposes of this Part, unless otherwise specifically defined or the context clearly requires a different meaning:

"Acceptance limits" means the data quality limits specified for analytical method performance.

"Accrediting authority" means the state or federal agency having the responsibility and accountability to grant accreditation to laboratories.

"Accuracy" means a measure of the degree of agreement between an observed value generated by a specific procedure and a true value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations.

"ASTM" means the American Society for Testing and Materials, West Conshohocken, PA a not-for-profit, voluntary standards development system.

"Analyte" means a chemical element, chemical compound, or physical property.

"Analyte of interest" means the chemical element, chemical compound, or physical property for which the laboratory is performing an analysis to determine the quantity in a sample for reporting pursuant to this Part.

"Analyzed reagents (AR)" means chemicals analyzed for impurities where the level of impurities is reported in accordance with specifications of the Committee on Analytical Reagents of the American Chemical Society.

"Analytical standard" means a solution of a compound or a mixture of compounds of known purity in an appropriate solvent used to prepare calibration standards. An Analytical standard may be traceable to NIST standard reference materials.

"Approved test methods" means the analytical methods specified in Section 186.180 of IEPA Part 186.

"ASTM E1301-95" means "Standard Guide for Proficiency Testing by Inter-laboratory Comparisons."

"Audit" means a thorough, systematic, qualitative examination of a laboratory for compliance with this Part, including but not limited to an examination of any of the following: facilities, equipment, personnel, training, procedures, documentation, record keeping, data verification, data validation, data management, data reporting, or any aspect of the laboratory's ability to meet the Agency's conditions for accreditation or comply with this Part.

"Bath" means one to 20 environmental samples of the same matrix that are prepared together with the same process and personnel, using the same lot of reagents with a maximum time between the start of processing of the first sample and start of processing of the last sample being 24 hours.

"Bias" means the systematic or persistent distortion of a measurement system which causes errors in one direction (the expected sample measurement is different from the true value).

"Blind sample" means a sub-sample for analysis with a composition known to the submitter that is used to test the analyst's, analyst-in-training's, or technician's proficiency in the execution of the measurement system. The analyst, analyst-in-training, or technician may know the identity of the sample but not its composition. The laboratory management may know the identity and composition of the blind sample.

"Calibrate" means initial calibration.

Appendix A (con't)

"Calibration blank" means a volume of distilled or deionized water containing the same reagents, solvents, acids or preservatives contained in the calibration standards. The calibration blank is used to determine the response of the instrument to the zero concentration of an analyte of interest.

"Calibration standard" means a solution of an analyte or mixture of analytes of known purity in an appropriate solvent used to calibrate the analytical instrument response with respect to analyte concentration.

"Certificate (certificate of approval)" means a document issued by the Agency to a laboratory that has met the criteria and conditions for accreditation as set forth in this Part. The certificate may be used as proof of accredited status. A certificate is always accompanied with a scope of accreditation.

"Certification" means accreditation.

"Certifying authority" means an accrediting authority.

"Chromatographic range" means the time frame over which analytes move out of the chromatography column.

"Confidence interval" means that range of values, calculated from an estimate of the mean and standard deviation, which is expected to include the population mean with a stated level of certainty.

"Continuing calibration verification (CCV) check" means the analysis of a continuing calibration verification check standard to determine the state of calibration of an instrument between recalibration, as required by Section 186.155 of IEPA Part186.

"Continuing calibration verification check standard" means a solution of an analyte or mixture of analytes of known purity in an appropriate solvent used to perform the continuing calibration verification check. The source of the analyte may be the same as the calibration standards' source or it may be a second source.

"Controlled access storage" means a refrigerator, cooler, rooms or building in which samples are held and from which samples may be removed only by authorized laboratory personnel.

"Corrective action" means an action taken by the laboratory to eliminate or correct the causes of an existing nonconformance in order to prevent the recurrence of the nonconformance.

"Corrective action plan" means a plan of corrective actions.

"Document" means any written or pictorial information describing, defining, specifying, reporting, or certifying any activities, requirements, procedures, or results.

"Drinking water" means water used or intended for use as potable water.

"Drinking water analyses" means analyses performed on water used or intended for use as potable water.

"Drinking water sample data" means analytical results generated by drinking water analysis.

"Environmental analyses" means measurement information results generated through the analyses of environmental samples.

"Environmental samples" means samples, excluding any laboratory generated quality control samples such as matrix spikes, duplicates, and laboratory control samples, for which the laboratory analytical results will be reported pursuant to IEPA Part 186.

Appendix A (con't)

"Environmental sample data" means measurement data generated through the analysis of environmental samples.

"EPA No. 600/8-91/213" means "Standard Operating Procedure for Lead in Paint by Hotplate or Microwave-Based Acid Digestions and Atomic Absorption or Inductively Coupled Plasma Emission Spectrometry."

"Evidentiary chain-of-custody" means the procedures and records which ensure that an intact, contiguous written record tracing the possession and handling of samples from the point that clean sample containers are provided by the laboratory or the point of sample collection through disposal are maintained.

"Final performance evaluation report" means a statement prepared by the USEPA or an Agency approved performance evaluation program that describes or evaluates a laboratory's performance after the laboratory's analyses of performance evaluation samples.

"Initial calibration" means the analyses of calibration standards for a series of different specified concentrations of an analyte of interest used to define the linearity and dynamic range of the response of the instrument to an analyte.

"Initial calibration verification (ICV) check" means analysis of an initial calibration verification check standard to determine the state of calibration of an instrument before sample analysis is initiated, as required by Section 186.155 of IEPA Part 186.

"Initial calibration verification check standard" means a solution of an analyte or mixture of analytes of known purity in an appropriate solvent used to perform the initial calibration verification check.

"Initial demonstration of method performance (IDMP) study" means the procedures performed by an analyst that insure that the analyst does not analyze unknown samples via a new unfamiliar method prior to obtaining experience as described in Section 186.160 of IEPA Part 186.

"Inorganic" means all parameters not included in organic parameters.

"LCB" means laboratory control blank (method blank).

"Laboratory control sample (LCS)" means an uncontaminated sample matrix with known quantities of analytes. T[illegible]lytes shall be obtained from a second source. The laboratory control sample is analyzed exactly like a sample to determine whether the measurement system is performing as expected using the evaluation procedures described in Section 186.160 of IEPA Part 186 and to determine whether the laboratory is capable of making accurate and unbiased measurements.

"Least precise step" means the part of the analytical procedure that results in the greatest error in measurement.

"Linear dynamic range (LDR)" means the range of concentrations over which the analytical system exhibits a linear relationship between the amount of material introduced into the instrument and the instrument's response.

"Litigation sample" means a sample, knowingly analyzed by the laboratory, for possible legal action.

"Matrix" means the predominant material of which the sample to be analyzed is composed. Sample matrices are:

"Aqueous" means any aqueous sample other than drinking water, potable water, or saline or estuarine water,

Appendix A (con't)

"Drinking water" means water used or intended for use as potable water,

"Non-aqueous liquid" means any organic fluid with <15% settleable solids;

"Saline or estuarine waters" means any aqueous sample from an ocean or estuary.

"Solids" means soils, sediments, sludges and other matrices with >15% settleable solids; or

"Chemical waste" means a product or by-product of an industrial process that results in a matrix not previously defined.

"Matrix spike (MS)" means an aliquot of matrix fortified (spiked) with known quantities of specific analytes and subjected to the entire analytical procedures in order to determine the effect of the matrix on an approved test method's recovery system.

"Matrix spike duplicate (MSD)" means a replicate matrix spike that is prepared and analyzed in order to determine the precision of the approved test method.

"Method" means a procedure or technique for performing an activity (for example sample preparation and sample analysis).

"Method blank (LCB)" means a sample which does not contain an analyte of interest above an acceptable level pursuant to Section 186.160 and which is processed simultaneously with and under the same conditions as samples being analyzed for analytes of interest.

"Method detection limit (MDL)" means the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix type containing the analyte. Unless specified by the approved test method, the method detection limit shall be determined using the procedures specified in Section 186.160 of IEPA Part 186.

"Megohm-cm" means megohm-centimeter.

"mg" means milligram.

"µmhos/cm" means micromhos per centimeter.

"Neat compound" means an undiluted compound.

"NIST" means the United Stated Department of Commerce, Technology Administration, National Institute of Standards and Technology (formerly National Bureau of Standards).

"Operating condition" means the state of the measurement system when samples are analyzed.

"Organic" means all analytes analyzed by all forms of gas chromatography and high pressure liquid chromatography (excluding ion chromatography).

"Parameter" means an analyte.

"Pattern of peak profile recognition for identification" means a series of chromatographic peaks used to identify multi-component analytes such as the aroclors, petroleum products, toxaphene and technical chlordane. The series of peaks used to identify a multi-component analyte have characteristic sizes, shapes and retention times.

Appendix A (con't)

"PE" means performance evaluation.

"Performance evaluation sample" means a sample prepared and supplied either by the Agency or an Agency approved performance evaluation program, whose composition is unknown to the laboratory management, analyst, analyst-in-training, and technicians. The performance evaluation sample is provided to test whether the laboratory can produce analytical results within specified performance limits.

"Performance evaluation study" means a single testing event within a performance evaluation program.

"Precision" means the measure of mutual agreement among individual measurements of a sample, usually under prescribed similar conditions, usually expressed as the standard deviation, variance, or range, in either absolute or relative terms.

"QC sample" means an independently purchased sample; with acceptance criteria supplied with the sample.

"Stable" means resistant to displacement or change.

"Standard operating procedure (SOP)" means a written, laboratory specific document which details the method of an operation, analysis or action whose techniques and procedures are thoroughly prescribed and which is accepted as the method for performing certain routine or repetitive tasks.

"Statistical outlier test" means a mathematical process for determining that an observation in unusually large or small relative to the other values in a data set.

"Surrogate" means an organic compound which is similar to the analytes of interest in chemical composition and behavior in the analytical process, but which is not normally found in environmental samples.

"Standard Methods" means Standard Methods for the Examination of Water and Wastewater, 18th edition, 1992.

"Traceability" means the property of a result of a measurement whereby it can be related to appropriate standards, usually international or national standards, through an unbroken chain of comparisons.

"True value" means the accepted or actual value of the quantity being measured.

"USEPA" means the United States Environmental Protection Agency.

"USEPA Water Pollution (WP) Performances Evaluation Study" means a performance evaluation program sponsored by the USEPA in which participation may be established by contacting the Illinois Environmental Protection Agency, Bureau of Water, Compliance Assurance, P.O. Box 19276, Springfield, Illinois 62794-9276.

USEPA Water Supply (WS) Performances Evaluation Study" means a performance evaluating program sponsored by the USEPA in which participation may be established by contacting the Illinois Environmental Protection Agency, Division of Laboratories, Quality Assurance Section, Environmental Laboratory Accreditation Program, P.O. Box 19276, Springfield, Illinois 62794-9276.

"Validation" means confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Validation is the process of examining a sample result to determine conformance with users' needs.

Verification" means confirmation by examination of and provision of objective evidence that specified requirements have been fulfilled. Verification is the process of examining a result of a given activity to determine conformance with this Part.

Appendix B – Sample Collection, Preservation, Shipment and Storage.

SAMPLING PROCEDURES

The following section states Teklab requirements on sample volumes, sample containers, sample preservation and holding times. See the Teklab field sampling procedures for information concerning field sampling. This section applies to samples received by Teklab and will be used to guide clients in sampling requirements specified in the "Federal Register, 40 CFR Part 136, Table II". Teklab does not reuse sample containers at this time. If sample containers are to be reused this section must be updated to include cleaning procedures for the containers.

SAMPLE CONTAINERS, PRESERVATION AND HOLDING TIMES

Total and prolonged preservation of samples is practically impossible. Preservation can only slow the changes that continue after the sample is removed from its source. Therefore as a general rule it is best to analyze the sample as soon as possible after collection. This is especially true when the parameter is expected to be in the low ug/l range. Table 2 lists recommended preservation and holding times, as well as, volumes need for analysis. See appropriate refrences for more information.

TABLE 2

SAMPLE VOLUME REQUIREMENTS, PRESERVATION AND MAXIMUM HOLDING TIMES

PARAMETER	VOL(ML)	CONTAINER	PRESERVATIVE	HOLDING TIME
PHYSICAL PROPERTIES				
Color	50	P,G	COOL, 4°C	48 Hours
Conductance	100	P,G	COOL, 4°C	28 Days
Hardness	100	P,G	HNO_3, pH<2	6 Months
Odor	200	G only	COOL, 4°C	24 Hours
pH	25	P,G	None Req.	Immed.
Residue				
TDS	100	P,G	Cool, 4°C	7 Days
TSS	100	P,G	Cool, 4°C	7 Days
TS	100	P,G	Cool, 4°C	7 Days
TVS	100	P,G	Cool, 4°C	7 Days
Settleable Matter	1000	P,G	Cool, 4°C	48 Hours
Temperature	1000	P,G	None Req.	Immed.
Turbidity	100	P,G	Cool, 4°C	48 Hours
METALS				
Dissolved	250	P,G	Filter on site HNO_3, pH<2	6 Months
Suspended	250	P,G	Filter on site	6 Months
Total	250	P,G	HNO_3, pH<2	6 Months
Chromium (+6)	250	P,G	Cool, 4°C	24 Hours
Mercury				
Dissolved	250	P,G	Filter HNO_3, pH<2	28 Days
Total	250	P,G	HNO_3, pH<2	28 Days
INORGANIC				
Acidity	100	P,G	Cool, 4°C	14 Days
Alkalinity	100	P,G	Cool, 4°C	14 Days
Boron	25	P only	Cool, 4°C	14 Days
Bromide	100	P,G	None Req.	28 Days
Chloride	50	P,G	None Req.	28 Days
Chlorine	200	P,G	None Req.	Immed.

TABLE 2 (CON'T)

PARAMETER	VOL(ML)	CONTAINER	PRESERVATIVE	HOLDING TIME
Cyanide	500	P,G	Cool, 4°C NaOH, pH>12 0.6 g ascorbic acid if CL_2 present	14 Days
Fluoride	500	P,G	None Req.	28 Days
Iodide	250	P,G	Cool, 4°C	24 Hours
Nitrogen				
Ammonia	500	P,G	Cool, 4°C H_2SO_4, pH<2	28 Days
Kjeldahl, Total	500	P,G	Cool, 4°C H_2SO_4, pH<2	28 Days
Nitrate & Nitrite	250	P,G	Cool, 4°C H_2SO_4, pH<2	28 Days
Nitrate	250	P,G	Cool, 4°C	48 Hours
Nitrite	250	P,G	Cool, 4°C	48 Hours
Dissolved Oxygen	300	G btl &top	None Req.	Immed.
Phosphorus				
Ortho, Dissolved	100	P,G	Filter on site Cool, 4°C	48 Hours
Hydrolyzable	100	P,G	Cool, 4°C H_2SO_4, pH<2	28 Days
Total	100	P,G	Cool, 4°C H_2SO_4, pH<2	28 Days
Total, Dissolved	100	P,G	Cool, 4°C H_2SO_4, pH<2 Filter on site	24 Hours
Silica	100	P only	Cool, 4°C	28 Days
Sulfate	500	P,G	Cool, 4°C	28 Days
Sulfide	500	P,G	Cool, 4°C 2 ml zinc acetate plus NaOH, pH>9	7 Days
Sulfite	100	P,G	None Req.	Immed.
ORGANIC				
BOD	500	P,G	Cool, 4°C	48 Hours
COD	50	P,G	Cool, 4°C H_2SO_4, pH<2	28 Days
Oil & Grease	1000	G only	Cool, 4°C H_2SO_4, pH<2	28 Days
Organic Carbon	250	P,G	Cool, 4°C H_2SO_4, pH<2	28 Days
Phenolics	1000	G only	Cool, 4°C H_2SO_4, pH<2	28 Days
MBAS	500	P,G	Cool, 4°C	48 Hours
NTA	100	P,G	Cool, 4°C	24 Hours
TOX	250	G only	Cool, 4°C H_2SO_4, pH<2	8 Days
VOA	40	G vials	Cool, 4°C	14 Days
Semi-Volatiles	250	G teflon	Cool, 4°C	14 Days
Pesticide/PCB/Herb	1000	G teflon	Cool, 4°C	14 Days

Appendix D – Pollution Prevention, Waste Management and References

ENVIRONMENTAL COMPLIANCE

Teklab will comply with all applicable federal, state and local laws concerning the handling and disposal of hazardous waste. Teklab will also comply with all applicable laws concerning the generation of air pollutant.

POLLUTION PREVENTION

Reagents and standards shall be purchased in quantities such that the entire quantity shall be used prior to expiration (where possible). Also, working standards and reagents shall be made up in quantities and/or concentrations that minimize the amount of waste generated.

Analysis shall use reagents which are the least environmentally damaging when a choice of reagents is permitted by the method. For example: a non-mercury containing reagent would be chosen over a mercury containing reagent, when either reagent is permitted in an analysis.)

Analysis shall use reusable labware in preference to disposable labware (where possible).

Solvents shall be recovered and recycled, when possible.

Paper products shall be recycled.

LABORATORY SAMPLE AND WASTE DISPOSAL

All laboratory samples and wastes are properly identified, disposed, or recycled according to applicable federal, state and local regulations. Records for sample disposal are kept indefinitely.

SOLVENTS: All solvents are incinerated or landfilled, except for freon, hexane and methylene chloride. Those solvents are recovered and recycled.

ACID WASTE: Acid waste is neutralized with a lime tank before being disposed of through the city of Collinsville wastewater treatment plant.

EXPIRED REAGENTS AND STANDARDS: All reagent and standards waste are disposed of via lab packs and are incinerated or landfilled as appropriate.

HAZARDOUS WASTE SAMPLES: Hazardous waste samples are either returned to the client (with appropriate documentation) or are lab packed and incinerated or landfilled as appropriate.

NON-HAZARDOUS SOLID SAMPLES: All solid samples are disposed of via lab packs and are incinerated or landfilled as appropriate.

NON-HAZARDOUS LIQUID SAMPLES: Non-hazardous wastewater, drinking water and groundwater are disposed of through the city of Collinsville wastewater treatment plant.

BIO-HAZARDOUS WASTES: All bio-wastes must be sterilized by autoclave at 121 deg. C for at least 30 minutes prior to disposal.

REFERENCES:

1. EPA 600/4-79-020 "Methods for Chemical Analysis of Water and Waste..".
2. "Standard Methods for the Examination of Water and Wastewater", 18th Edition.
3. USEPA SW-846 "Test Methods for Evaluating Solid Waste", Physical/Chemical Methods, 3rd Edition.
4. Illinois EPA Title 35, Subtitle A, Chapter II, Part 186.
5. "Quality Assurance for Chemical Measurements" Taylor, from Lewis Publishers.
6. 40 CFR

BIOCHEMICAL OXYGEN DEMAND

First Environmental Laboratories - Bench Reference

Biochemical Oxygen Demand (BOD): EPA Method 405.1 (Probe Method)
Preservation Requirements: Cool 4°C
Single Analysis Sample Volume: 300mL minimum
Holding Time: 48 Hours
Reporting Limit: 1 mg/L

Determinative Procedure

The sample of waste, or an appropriate dilution, is incubated for 5 days at 20C I the dark. The reduction in dissolved oxygen concentration during the incubation period yields a measure of the biochemical oxygen demand.

Preparation of Dilution Water:

1. Fill dilution water container with desired amount of de-ionized water. (Use a minimum of 3L)
2. Add one nutrient buffer pillow for every 3 L of D.I. water.
3. Saturate water with oxygen by shaking vigorously. Bring water to a temperature of 20 C prior to use.

Preparation of Seed:

1. Add one Polyseed capsule to a beaker containing 500mL DI water. Stir for at least 30 minutes.

Procedure:

1. Sample pH must be between 6.5-7.5 prior to analysis. Any residual chlorine must be removed prior to analysis
2. Add 2 mL seed to each BOD bottle. Add 3mL and 6mL to the seed control bottles.
3. Add desired quantity of sample to a half filled 1L graduated cylinder. Record volume used in BOD logbook.Carefully pour into a 300mL BOD bottle. Read D.O. using the calibrated YSI meter. (See manufacturers documentation for proper calibration procedures). At a minimum, perform 2 different dilutions for each sample.
4. Incubate in the dark for 5 days at 20 C.
5. Read DO after incubation period. A residual D.O. of 1 mg/L and an uptake of at least 2 mg/L is required for results to be considered acceptable.

Calculation

$$\text{BOD, mg/L} = \frac{(D1 - D2) - (B1 - B2)f}{P}$$

Where:

D1 = DO of diluted sample immediately after preparation, mg/L
D2 = DO of diluted sample after 5 day incubation period, mg/L
P = Decimal volumetric fraction of sample used
B1 = DO of seed control before incubation, mg/L
B2 = DO of seed control after incubation period, mg/L
f = volume of seed in diluted sample/volume of seed in seed control.

Reference SOP: BOD

QCIs

QCI	Preparation	Frequency	Criterion
Dilution water blank	NA	Per batch	<0.2 mg/L
Seeded dilution water	NA	Per batch	Between 0.6-1.0 mg/L
Standard, low level (20 mg/L)	Dilute to appropriate range	per batch	17-23 mg/L
Standard, high level (200 mg/L)	Dilute to appropriate range	per batch	180-220 mg/L

Reagents

Seed: Polyseed BOD seed inoculum, purchased. Cat No. P-110

Nutrient Buffer: BOD nutrient buffer pillows, purchased. Hach Cat. No. 14861-98

Standards

Glucose-Glutamic Acid Check Standard: Weigh 150mg of glucose and 150mg glutamic acid, and add to a 1000 mL volumetric flask. Dilute to 1000 mL/. This solution has a BOD value of 200 mg/L. Prepare a 20 mg/L check by diluting this solution 1:10.

Reference SOP: BOD

NITRATE

First Environmental Laboratories

Standard Operating Procedure

Title: Nitrate + Nitrite, Cadmium Reduction Using HACH Reagent, Manual Colorimetric Determination

Regulatory References: EPA 600/4-79-020 Method 353.3 Modified
Note: Nitrate by Ion Selective Electrode is the approved method for the analysis of samples in accordance with the Safe Drinking Water Act.

Regulatory Limits: SDWA 10.0 mg/L

Preservation Requirements: H_2SO_4 to pH<2 (approximately 2 mL of 1 + 1 acid), Cool 4°C

Container: 250 cc plastic

Single Analysis Sample Volume: 25 mL

Holding Time: 28 days for preserved waste water and chlorinated drinking water
14 days for preserved non-chlorinated drinking water
48 hours for non-preserved, non-chlorinated drinking water

(Range) Reporting Limit - Upper End: 0.05 mg/L - 2.00 mg/L

Summary of Method: Nitrate (NO_3^-) is reduced almost quantitatively to nitrite (NO_2^-) in the presence of cadmium (Cd). The nitrite ion reacts in an acidic medium with sufanilic acid to form an intermediate diazonium salt which couples to gentistic acid to form an amber-colored product. The HACH powdered reagent (NitraVer 5 Nitrate Reagent) is a modification of the reagent specified in Standard Methods, 18th Edition, Method 4500-NO_3^- Cadmium Reduction Method. The HACH reagent uses gentistic acid in place of 1-naphthylamine. The HACH procedure is not approved by the regulating agencies. Use of the procedure requires appropriate notation of modification when citing method references.

This method detects nitrate + nitrite. In order to express the result as only nitrate, a correction is made for the nitrite present in the sample by analyzing for nitrite and subtracting the amount found.

1. Instrumentation / Apparatus / Glassware

1.1. Spectrophotometer, for use at 400 nm providing a light path of 1 cm.

2. Reagents

All reagents should be prepared using deionized water and volumetric glassware.

2.1. HACH NitraVer 5 Powder Pillow for 25 mL sample volume: Purchased. HACH No. 14034-99 (100 pp / box)

2.2. Phenolphthalein indicator:

2.2. 6N Sodium Hydroxide: Cautiously dissolve 240g of NaOH pellets in DI water. Cool and dilute to FV of 1L volumetrically. Expiration Date: 1 year.

2.3. 1N H_2SO_4: dilute 28 mL of concentrated H_2SO_4 to 1 L with DI water volumetrically.

2.4. 5N H_2SO_4: dilute 140 mL of concentrated H_2SO_4 to 1 L with DI water volumetrically.

3. Standards

3.1. 100 mg/L Nitrate Stock Standard: Dissolve 0.7218g anhydrous KNO_3, which has been dried for 24 hours in a 103-105°C oven, in DI water and dilute to FV of 1L volumetrically. Preserve with 2 mL of chloroform. Expiration Date: 6 months

3.2. 2.00 mg/L LCS: Pipet 2 mL of stock standard into a 100 mL volumetric and dilute to FV with DI water. Expiration Date: Prepare fresh daily.

3.3. 1.00 mg/L CCVS: Pipet 2 mL of stock standard into a 100 mL volumetric and dilute to FV with DI water. Expiration Date: Prepare fresh daily.

3.4. To Spike the Sample: Pipet 200 uL of stock standard into 25 mL of the sample being spiked. The sample is now spiked with 0.80 mg/L of nitrate.

3.5. Recommended source for ICVS / PE is APG

3.6. Calibration Curve

3.6.1. Purpose: A calibration curve relates instrument response to sample concentration.

It also proves that the instrument response, over a determined concentration range, can be predicted using a mathematical equation.

3.6.2. Spectrophotometric analyses require the construction of a calibration curve. **First Environmental Laboratories, Inc.** utilizes a referenced curve system, e.g., a single continuing calibration standard is used to verify the curve on a daily basis.

A standard curve to be referenced requires a blank and five standards evenly distributed throughout the range of the analysis. The data must be collected under the same conditions as those that will exist during routine analyses. Two set of data are generated, each on different days. The results of the calculations generated from each data set are averaged.

3.6.3. Prepare a standard curve by plotting the absorbance values of standards (y-axis) versus the corresponding concentrations (x-axis). Inclusion of the blank in the curve is permitted if the actual instrument response from the blank is used as a data point for constructing the curve. If the spectrophotometer is set to zero with the blank, or if the blank is subtracted out prior to calculating the final result, do not include the blank in the curve. In this instance, including the blank would artificially force the curve through zero.

3.6.4. Frequency: A new calibration curve must be prepared at least yearly. Changes in instrument or reagent conditions, which result in failure to obtain an acceptable response for the CCVS, would indicate the need to prepare a new curve. If more than one analyst performs the analysis, each analyst does not need to have separate curves provided they are able to obtain acceptable results on the CCVS.

3.6.5. Acceptance Criterion: A correlation coefficient ≥ 0.995 must be achieved using all calibration standards. The ICVS must be analyzed immediately following the curve. The result must be within $\pm$ 10% of the true value or within the acceptance ranges established according to the manufacturer of the ICVS. The narrower of the two should normally be applied.

3.6.6. Corrective Action: Since the calibration curve is used for calculating results for all samples and quality control indicators, a curve meeting all requirements must be established prior to analyzing client samples.

Care should be taken when choosing the concentrations of the standards for the calibration curve. If the intercept is large or if the correlation coefficient is poor, then the concentration of the standards used in relation to the detection limit and linear range should be carefully re-evaluated.

Rule of Thumb: Each standard on the curve should back calculate to within $\pm$ 10% of the true value.

3.6.7. Documentation Requirements: The raw data and calculations supporting the use of a referenced curve must be easily retrieved. Record the response factor used to perform the calculations, as well as the reference notation for curve raw data, with the raw data supporting the analysis.

3.6.8. Prepare Calibration Curve: Prepare 0.50, 1.00, 1.50, 2.00, and 2.50 mg/L NO3 standard solution by diluting 0.50, 1.00, 1.50, 2.00, and 2.50 mL of stock solution to 100 mL with deionized water.

4. Analytical Procedures

4.1. Interferences

4.1.1. Strong oxidizing and reducing substances will interfere. Ferric iron causes high results and must be absent. Chloride concentrations above 100 mg/L will cause low results.

4.1.2. Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment. Pretreatment consists of neutralizing the sample with sodium hydroxide prior to beginning the analysis.

4.2. Sample Preparation

4.2.1. Total Soluble Nitrate can be determined by solubilizing an aliquot of sample in deionized water, separating the liquid from the solid, and analyzing the liquid phase for nitrate. Use a ratio is 2g sample : 48 mL deionized water. The liquid can be separated from the solid by centrifuging or by allowing the sample to settle over night. Calculate the final result by multiplying the mg/L nitrate found in the liquid phase by the dilution factor. The final result should be converted to a dry weight basis.

4.2.2. If an unpreserved aqueous sample contains particulates, filter the sample through a Whatman No. 40 filter. Alternatively, a background reading may be obtained on an acidified aliquot of sample.

4.3. Determinative Procedure

4.3.1. All samples should be brought to room temperature prior to beginning the analysis. All sample must be neutralized prior to beginning the analysis.

4.3.2. A 25 mL aliquot of DI water processed through the preparative and colorimetric procedure serves as the procedure blank and monitors the sample preparation for contamination. Frequency: At the beginning and end of the run, and after every 20th sample.

4.3.3. A 25 mL aliquot of 2.00 mg/L CCVS processed through the preparative and colorimetric procedure monitors the sample preparation for analyte loss and/or contamination. Frequency: At the beginning and end of the run, and after every 20th sample.

4.3.4. A 25 mL aliquot of DI water processed through the colorimetric procedure serves as the reagent/calibration blank and monitors the reagents for contamination as well as, the instrument baseline or zero. Frequency: At the beginning and end of the run, and after every 10th sample.

4.3.5. A 25 mL aliquot of 1.00 mg/L CCVS processed through the colorimetric procedure monitors the calibration of the instrument. Frequency: At the beginning and end of the run, and after every 10th sample.

4.3.6. Duplicate spiked aliquots of sample (MS/MSD) monitor the precision and accuracy of the procedure for a specific matrix. If the sample required preparation, the MS/MSD are processed in a similar manner. Frequency: 1/20 samples or less of the same matrix. See section 3.4. for instructions on how to spike the sample.

4.3.7. Set the wavelength to 400 nm on the spectrophotometer.

4.3.8. Pour approximately 50 mL of DI water blank, LCS, and samples into a 150 mL beaker cup.

4.3.9. Add 2-3 drops of phenolphthalein indicator.

4.3.10. Add 1 mL 6N NaOH. Add dropwise until pink color remains.

4.3.11. Discharge the pink color to clear with the dropwise addition of 5N and 1N H_2SO_4.

4.3.12. If the sample has developed a precipitate, filter through a Whatman #40.

4.3.13. Measure 25 mL of blanks, standards, and sample into a disposable culture tube using a graduated cylinder.

4.3.14. Add the contents of one powder pillow to each tube.

4.3.15. Stopper the tubes. A one minute reaction period is required. Samples are vigorously shaken for 1 minute.

4.3.16. After shaking the last sample, allow the samples to react and additional five minutes without inverting the tubes.

4.3.17. Remove the stoppers from the tubes. Zero the instrument on the reagent blank for best results. The blank absorbance should be monitored for trends. Read the absorbance of each tube @ 400 nm.

4.3.18. If the sample is colored or remains turbid, obtain background readings on acidified sample.

4.4. Calculation

Include factors resulting from sample preparation and/or the need to analyze the sample at a dilution. The formula provided below assumes the sample was a clean water not requiring dilution.

mg/L NO_3^- as N = abs of sample x response factor
where: response factor = concentration of standard (mg/L) = slope
absorbance of standard

4.5. Expression of Results for Solid Samples

Soils should be expressed on a dry weight basis as mg/kg. The exception would be a solid material submitted for RCRA compliance, in which case the results should be expressed on a wet weight basis.

Convert wet weight results to dry weight by dividing the wet weight result by the decimal equivalent of the percent total solids.

5. Quality Control

Quality Control Indicator (QCI)	Frequency	Control Limit (Interim)	Control Limit (Statistical)
Calibration Curve	**Referenced**	**cc = 0.995, back calculation of percent recovery with 10% of true value**	**NA**
Initial Calibration Verification	**Immediately following the curve**	**± 10% or within stated limits**	**As supplied by manufacturer**
Reagent Blank / Continuing Calibration Blanks	**Daily at the beginning, end, and after every 10 samples**	**Less than the reporting limit**	**NA**
Continuing Calibration Standard (CCVS) - concentration upper end or middle of range	**Daily at the beginning, end, and after every 10 samples**	**± 10%**	**3 standard deviations for the control limit and 2 standard deviations for the warning limit***
Procedure Blank (PB)	**Per batch, where a batch is defined as daily or every 20 samples - whichever is less**	**Less than the reporting limit**	**NA - unless a contamination problem exists - then 3 standard deviations for the control limit and 2 standard deviations for the warning limit**
Laboratory Control Standard (LCS) - concentration upper end of range	**Per batch, where a batch is defined as daily or every 20 samples - whichever is less**	**± 20%**	**3 standard deviations for the control limit and 2 standard deviations for the warning limit***

Matrix Spike / Matrix Spike Duplicate (MS/MSD) - concentration 20 to 50 percent of range	**Per batch, where a batch is defined as daily or every 20 samples - whichever is less**	**±25%**	**3 standard deviations for the control limit and 2 standard deviation for the warning limit**

6. Notes

6.1. Tips and Hints

6.1.1. Shaking time and technique influence color development. Maintain consistent technique for the best results.

6.1.2. A deposit of unoxidized metal will remain after the NitraVer 5 Nitrate Reagent Powder dissolves and will have no effect on test results.

6.1.3. An amber color will develop if nitrate nitrogen is present.

6.2. Discussion - Regulatory Compliance

The use of HACH reagents for this method does not have EPA approval and does not exactly meet the requirements of the cadmium reduction method. It is a modified procedure.

The following is a breakdown of the method approvals according to NPDES regulations and Drinking Water Regulations:

Colorimetric Brucine(352.1): EPA has proposed to delete this method from the list of approved methods and it is not recommended for either wastewater and drinking water analysis.

Spectrophotometric, cadmium reduction (353.3): The assumption is manual reduction using a column. This method is approved for both wastewater and drinking water analysis, but if the actual apparatus is utilized, it is extremely time consuming and inefficient.

Automated hydrazine reduction(353.1): This method is approved for both wastewater and drinking water analysis but requires an automated instrument.

Automated cadmium reduction (353.2): This method is approved for both wastewater and drinking water analysis but also requires an automated instrument. This method seems to be the most reliable and flexible.

Ion Selective Electrode (WeWWG/5880): The method reference is from an for a Form from Orion which is obsolete and no longer in print. This method was approved for use effective 30 July 1993 for drinking water analysis. **It is not approved for wastewater analysis and it will probably not be approved in the future due to exhibited interferences.** Chloride is a known interference. Chloride is likely to exist at high concentrations in wastewaters. Whether or not ISE can be used for groundwater monitoring samples not associated with an NPDES permit is a vague point.

Ion Chromatography (300.0): This method is approved for drinking water analysis. If the sample is not preserved, then the holding time reverts to 48 hours. The sulfuric preservative is a problem if the simultaneous analysis of sulfate is desired. The method is not approved for wastewater analysis, but it has been recommended for approval by the methods group at EMSL.

6.3. Non-Regulatory References

6.3.1. HACH Water Analysis Handbook, 1989 Hach Chemical Company, page 396-400.

6.3.2. HACH Procucts for Analysis, 1993-1994.

7. Approvals

Reviewed for Technical Accuracy by:_____

Reviewed for Quality Assurance Compliance by:_____

Implementation Date: _____

End Use Date: _____

SULFIDE

First Environmental Laboratories

Standard Operating Procedure

Title: Sulfide, Total - Methylene Blue Colorimetric Determination

Regulatory References: EPA 600/4-79-020, Method 376.2
Standard Methods, 19th Edition, Method 4500-S_2-A,C,D

Regulatory Limits: Analysis for total sulfide may be substituted for reactive concentration if total sulfide concentration is equal to or less than 10 ppm. If the total concentration is greater than 10 ppm, then the reactive procedure must be performed.

Preservation Requirements: Aqueous samples are preserved to pH>9 with approximately 1 mL of 6N sodium hydroxide/2N zinc acetate/sodium hydroxide preservative per 250cc sample. The sample container should be filled completely with a minimum of aeration. Solid samples are not preserved. All samples are kept cool at 4°C.

Container: 250 cc plastic for aqueous samples and 4 or16 oz wide mouth jars for solid samples.

Single Analysis Sample Volume: 10 mL or 10g

Holding Time: 7 days

(Range) Reporting Limit - Upper End: 0.05 mg/L – 1.00 mg/L
The range may be extended by diluting the sample.

Summary of Method: This method is based on the reaction of sulfide, ferric chloride, and dimethyl-p-phenylenediamine to produce methylene blue. Ammonium phosphate is added after color development to remove ferric chloride color. The absorbance is measured by a spectrophotometer set at 625 nm to determine the quantity of sulfide present.

Acid insoluble sulfides are not measured by the use of this test. Copper sulfide is the only common sulfide in this class.

Another method of analysis, back titration using excess iodine, is used to standardize the stock solution and to analyze samples that have sulfide content greater than 10 mg/L. A separate SOP details the titrimetric procedure. A separate SOP details the preparation of solids using distillation.

1. Instrumentation / Apparatus / Glassware

1.1. Spectrophotometer having a 1 cm cell length

1.2. Magnetic Stirrer and Stir Bars

1.3. Analytical balance capable of accurately weighing 0.0001g

1.4. Distillation apparatus having a Graham Condenser and 500-1000 mL flask.

2. Reagents

All reagents should be prepared using deionized water and volumetric glassware.

2.1. Zinc Acetate, 2N: Purchased Expiration Date: per manufacturer's instructions.

2.2. Zinc Acetate, 0.2N: dilute 100 mL of purchased 2N ZnAc to 1L fv with DI water. Expiration Date: 1 year

2.3. Sodium Hydroxide, 6N: Dissolve 240g of NaOH pellets in DI water and dilute to FV of 1L volumetrically. Expiration Date: 1 year

2.4. Zinc Acetate/Sodium Hydroxide Preservative: mix equal volumes of 2N zinc acetate and 6N sodium hydroxide.

2.5. Sulfide 1 Reagent: Purchased from HACH, Catalog No. 1816-14

2.6. Sulfide 2 Reagent: Purchased from HACH, Catalog No. 1817-14

3. Standards

3.1. 1000 mg/L Sulfide Stock Standard: Dissolve 7.45g sodium sulfide nanohydrate (Na_2S * 9 H_2O) in a beaker of DI water containing 20 mL 2N zinc acetate and 2 mL 6N sodium hydroxide. The zinc acetate will cause the sulfide to precipitate as it dissolves preventing loss of sulfide. Due to formation of the ZnS precipitate, the solution will have a milky white appearance. Transfer to a volumetric flask and rinse the beaker several times adding the rinsings to the flask. Dilute to a final volume of 1L. When weighing the sodium sulfide use only the crystals, do not use any liquid. Do not use metal utensils during the preparation of the stock standard. Perform the preparation as quickly as possible to prevent loss of sulfide. This solution is standardized using the titrimetric procedure described in the titrimetric sulfide SOP. Use 10 - 25 mL of standard in place of 200 mL of sample. If the solution has been prepared properly the concentration will be in

the range of 920-980 mg/L. Expiration Date: Undetermined. Standardize the stock solution monthly. See the SOP for Sulfide, Back Titration Using Excess Iodine. Refridgerate!

Exceptional Contact Hazard!. Read the MSDS.

3.2. 100mg/L Sulfide Intermediate #1 Standard: Add 1 mL zinc acetate/sodium hydroxide preservative to 50 mL of DI water. Mix the stock sulfide standard well on a magnetic stirrer and while mixing withdraw and appropriate aliquot. Add to the flask containing the preservative and bring to FV of 100 mL volumetrically. Expiration Date: Daily
Note: Keep standard capped whenever possible.

Determine the appropriate amount of stock standard required to obtain 100 mL of intermediate sulfide standard as follows:

$$\text{mL of stock needed} = \frac{1000}{\text{concentration (ppm) from standardization of stock solution}} \times 10$$

Do not use reduced volumes when preparing the intermediate standard. Since the stock solution is a suspension, use of smaller volumes leads to inaccuracy.

3.3. 10 mg/L Sulfide Intermediate #2 Standard: Pipet 10 mL of 100 mg/L intermediate #1 standard directly into 100 mL of DI water containing 1 mL of zinc acetate/sodium hydroxide preservative.

3.4. 1 mg/L CCVS: Pipet 1 mL of intermediate #2 standard directly into 9 mL of DI water. (Note: The CCVS is at the upper end of the linear range due to the unstability of the sulfide standard.)

3.5. To Spike the Sample: Pipet 200 uL of intermediate standard directly into 10 mL of the sample being spiked. The sample is spiked with 0.20 mg/L of sulfide.
Note: prepare the spike immediately prior to adding the colorimetric reagents.

3.6. Recommended source for ICVS and PE is ERA.

3.6. Calibration Curve

3.6.1. Purpose: A calibration curve relates instrument response to sample concentration. It also proves that the instrument response, over a determined concentration range, can be predicted using a mathematical equation.

3.6.2. Spectrophotometric analyses require the construction of a calibration curve. **First Environmental Laboratories, Inc.** utilizes a referenced curve system, e.g., a single continuing calibration standard is used to verify the curve on a daily basis.

A standard curve to be referenced requires a blank and five standards evenly distributed throughout the range of the analysis. The data must be collected under the same conditions as those that will exist during routine analyses. Two set of data are generated, each on different days. The results of the calculations generated from each data set are averaged.

3.6.3. Prepare a standard curve by plotting the absorbance values of standards (y-axis) versus the corresponding concentrations (x-axis). Inclusion of the blank in the curve is permitted if the actual instrument response from the blank is used as a data point for constructing the curve. If the spectrophotometer is set to zero with the blank, or if the blank is subtracted out prior to calculating the final result, do not include the blank in the curve. In this instance, including the blank would artificially force the curve through zero.

3.6.4. Frequency: A new calibration curve must be prepared at least yearly. Changes in instrument or reagent conditions, which result in failure to obtain an acceptable response for the CCVS, would indicate the need to prepare a new curve. If more than one analyst performs the analysis, each analyst does not need to have separate curves provided they are able to obtain acceptable results on the CCVS.

3.6.5. Acceptance Criterion: A correlation coefficient ≥ 0.995 must be achieved using all calibration standards. The ICVS must be analyzed immediately following the curve. The result must be within $\pm$ 10% of the true value or within the acceptance ranges established according to the manufacturer of the ICVS. The narrower of the two should normally be applied.

3.6.6. Corrective Action: Since the calibration curve is used for calculating results for all samples and quality control indicators, a curve meeting all requirements must be established prior to analyzing client samples.

Care should be taken when choosing the concentrations of the standards for the calibration curve. If the intercept is large or if the correlation coefficient is poor, then the concentration of the standards used in relation to the detection limit and linear range should be carefully re-evaluated.

Rule of Thumb: Each standard on the curve should back calculate to within $\pm$ 10% of the true value.

3.6.7. Documentation Requirements: The raw data and calculations supporting the use of a referenced curve must be easily retrieved. Record the response factor used to

perform the calculations, as well as the reference notation for curve raw data, with the raw data supporting the analysis.

3.6.8. Prepare Calibration Curve: Prepare 0.125, 0.250, 0.500, 0.750, and 1.00 mg/L Sulfide standard solutions by diluting 0.125, 0.250, 0.500, 0.750, and 1.00 mL of stock solution to deionized water containing 1 mL of zinc acetate/sodium hydroxide preservative solution. Dilute to 100 mL.

4. Analytical Procedures

4.1. Interferences

4.1.1. Strong reducing agents, including thiosulfate, sulfite, and various organic compounds, both solid and dissolved, interfere in the procedure by preventing formation of the blue color.

4.1.2. Thiosulfate at concentrations of about 10 mg/L may retard color formation or completely prevent it. Sulfide itself prevents the reaction if its concentration is very high, in the range of 200 mg/L. If sample has a strong sulfide odor, use a smaller sample aliquot.

4.1.3. Samples must be taken with a minimum of aeration in order to avoid volatilization of sulfides and reaction with oxygen, which may convert sulfide to unmeasurable forms.

4.2. Determinative Procedure

4.2.1. A 10 mL aliquot of DI water processed through the colorimetric procedure serves as the reagent/calibration blank and monitors the reagents for contamination.. Frequency: Daily at the beginning and of the run

4.2.2. A 10 mL aliquot of 1.00 mg/L CCVS processed through the colorimetric procedure monitors the determinative procedure. Frequency: At the beginning and end of the run, and after every 10th sample. Section 3.4. tells you how to prepare the CCVS.

4.2.3. Duplicate spiked aliquots of sample (MS/MSD) monitor the precision and accuracy of the procedure for a specific matrix. If the sample required preparation, the MS/MSD are processed in a similar manner. Frequency: 1/20 samples or less of the same matrix. Section 3.5. tells you how to spike the sample.

4.2.4. Shake the sample gently and quickly withdraw an aliquot for analysis before the precipitate settles.

4.2.5. Pipet 10 mL of sample into a disposable beaker cup or test tube.

4.2.6. Add 0.4 mL Sulfide 1 Reagent to the beaker cups/test tube. Mix well.

4.2.7. Add 0.4 mL Sulfide 2 Reagent to the beaker cups/test tube. Mix well.
(Note: This reagent is a skin irritant. Read the label!)

4.2.8. After 5 minutes, read absorbance on the spectrophotometer at 625 nm.

4.2.9. If background color is suspected to contribute to the final absorbance reading, process a separate aliquot of sample through all steps with the exception of the addition of Sulfide 2 Reagent.

4.3. Calculation

4.3.1. The calculation is based on a five point referenced curve, which is validated at the time of preparation with an initial calibration verification standard. For details regarding the preparation of calibration curves, see the Calibration Curves SOP.

4.3.2. Include factors resulting from sample preparation and/or the need to analyze the sample at a dilution. The formula provided below assumes the sample was a clean water not requiring dilution.

4.3.3. If necessary, correct the absorbance reading by subtracting the background reading. From the corrected absorbance, determine the mg/L of sulfide present by reference to the calibration curve.

$$\text{mg/L sulfide} = \text{abs of sample} \times \text{response factor}$$

where: $$\text{response factor} = \frac{\text{concentration of standard (mg/L)}}{\text{absorbance of standard}} = 1/\text{slope}$$

The slope is obtained from the calculation of the correlation coefficient, where m = slope. This calculation is based on he relationship, $x = my + b$

4.4. Expression of Results for Solid Samples

Soils should be expressed on a dry weight basis as mg/kg. The exception would be a solid material submitted for RCRA compliance, in which case the results should be expressed on a wet weight basis.

Convert wet weight results to dry weight as follows:
divide the wet weight result by the decimal equivalent of the % total solids.

5. Quality Control Indicator Assessment

Each quality control indicator (QCI) must be assessed for compliance with the following criterion on a real time basis. Failure to meet the criterion fails the batch. Record the percent recovery of QCIs and/or the relative percent difference (RPD) on the raw data/lab book form.

Quality Control Indicator (QCI)	Frequency	Control Limit (Interim)	Control Limit (Statistical)
Reagent Blank / Continuing Calibration Blanks	**At the beginning, end, and after every 10th sample**	**Less than the reporting limit**	**NA**
Continuing Calibration Verification Standard (CCVS) - concentration upper end of range	**At the beginning, end, and after every 10th sample**	**± 15%**	**3 standard deviations for the control limit and 2 standard deviations for the warning limit***
Matrix Spike / Matrix Spike Duplicate (MS/MSD) - concentration 20 to 50 percent of range	**Per batch, where a batch is defined as twenty samples or less**	**±25%, RPD ± 20**	**3 standard deviations for the control limit and 2 standard deviation for the warning limit**

6. Notes

6.1. Tips and Hints

Avoid excessive agitation of sample prior to and during analysis.

Follow the instructions for the preparation of the stock standard explicitly. Use of both the zinc acetate and sodium hydroxide is critical.

6.2. Discussion - Regulatory Compliance

6.3. Non-Regulatory References

6.3.1. Water Analysis Handbook, HACH 1989.

7. Approvals

Reviewed for Technical Accuracy by: _____

Reviewed for Quality Assurance Compliance by: _____

Implementation Date: _____

End Use Date: _____

METHANE / ETHANE / ETHENE

Analyte: Methane/Ethane/Ethylene
Matrix: Water

Date Prepared: 4/10/89
By: JMB
Microbac Method:
Revision Date: 5/21/98 BJJ
Approved By:

Reference: ASTM D-1945/D-3588

Method Summary: This procedure quantitates the amount of methane (CH_4), ethane (C_2H_6) and ethylene (C_2H_4) in aqueous samples by measuring the concentration of the gases found in the headspace of the sample.

Equipment Needed:

Tedlar bags
MTI portable GC Twin TCD Detectors & computer
EZ Chrom Software
Wrenches - various sizes
Regulator - able to regulate from 1000 psi down to 20 psi
Various tubes and pipe fittings
Teflon tape
One qt. ball jar with hole in lid and tape over hole and/or 250ml amber bottles equipped with Teflon septa
Needle assembly

Reagents Needed:

Gas standards: UHP Helium (carrier gas)
Methane/Ethane/Ethylene Standards at various concentrations

Sample handling: No preservatives are required for this procedure. The samples should be stored at 4° C and can be held for up to 1 month before analysis. Collect sample in 2-250 ml amber glass bottles equipped with Teflon septa. No headspace.

Special Precautions on Procedure:

1) No smoking, sparks, or flame in area
2) If gas is wet, do not shoot into column:
   -dry sample loop
   -attach filter between sample container and G.C.
3) Make sure all connections are air-tight
4) Pressure of gas must be exactly the same for STD and samples, i.e. either both out of bags or both out of pressurized tanks
5) Keep needle guard on needle to prevent accidental puncture wounds (when needle not in use).
6) Only the headspace gas is injected into the G.C. - do not inject any water.

Analyte: Methane/Ethane/Ethylene
Matrix: Water

Date Prepared: 4/10/89
By: JMB
Microbac Method:
Revision Date: 5/21/98 BJJ
Approved By:

Procedure (for details, see reference):

1) Set G.C. to proper operating conditions:

Column A: OV-1, 4m, 1.2dF, 0.15mm ID, 0.25mm ID

Column temp.	35 degrees C
Sampling time	30S
Inject time	40ms
Run time	180S
Detector filament	on
Autozero	on
Detector sensitivity	high
Column head pressure	23.0 psi

Column B: Poraplot U, 6m, 0.32mm ID

Column temp.	55 degrees C
Sampling time	30S
Inject time	40ms
Run time	180S
Detector filament	on
Autozero	on
Detector sensitivity	high
Column head pressure	23.0 psi

Integration Method: (Refer to software manual on how to build method)

Configuration

Instrument	1
Board	1
Number of detectors	2
Control	None
Ready signal	1 (normally closed)
Trigger signal line	2
Stop signal line	0

Channel A & B

Name	TCD
Connected to analog input #	1 (2 for B)
Yaxis labeled	Volts
Yaxis multiplier	0.000001
Maximum # of peaks	25
Maximum # of named peaks	25

Analyte: Methane/Ethane/Ethylene
Matrix: Water

Date Prepared: 4/10/89
By: JMB
Microbac Method:
Revision Date: 5/21/98 BJJ
Approved By:

Acquisition

Board #	1
Base address (Hex)	310
Interrupt level	IRQ 10 (or IRQ 11)
Base frequency	10
Range	D-IV

To Calibrate:

1) Analyze mid-point standard under an old method
2) Identify peaks for new method
3) Calibrate method using the following standards: 0.005%, 0.01%, 0.1%, 0.5%, 1%, 10% (see EZ Chrom SOP to build method)

Once the method is calibrated, analyze a reference standard.

NOTE: Reference must be $\pm$ 2% unnormalized. If reference fails, re-analyze. If reference fails a second time, recalibrate.

Sample Analysis:

1) Allow samples to reach room temperature. If a 1 quart ball jar is being used and there is adequate headspace (>50 ml), no further prep is required. Proceed to Step #3.
2) If a 250ml amber bottle is being used and there is no headspace, remove 50ml of water with a syringe. Proceed to Step #3.
3) Shake sample vigorously (upside down) for 30 seconds.
4) Attach needle to injection port.
5) Insert needle through hole in tape or septa.
6) Push start button.
7) When sampling is finished, remove needle from sample and attach needle guard. Place a piece of tape over puncture hole on jar.
8) When analysis is complete, method will automatically integrate. Expand chromatogram of channel 2 and confirm proper integration of N_2 and CH_4. Select an external standard report and record percentages (see attached data sheet).

Analyte: Methane/Ethane/Ethylene
Matrix: Water

Date Prepared: 4/10/89
By: JMB
Microbac Method:
Revision Date: 5/21/98 BJJ
Approved By:

9) Measure the volume of water and the total volume of the container. Calculate the volume of the headspace and then the % CH_4 in headspace:

$$\frac{\text{ml headspace}}{\text{ml } H_2O} \times 6.6 = \frac{\text{mg } CH_4}{\text{liter } H_2O}$$

NOTE: 6.6 is correction factor for methane
12.4 is correction factor for ethane/ethylene

10) Record data on queue list.
11) File data sheets.

Compounds of Interest:

Column A:

Compound	RT
Isobutane	0.11
N-Butane	0.12
Isopentane	0.18
N-Pentane	0.21
Hexanes	

Column B:

Compound	RT	MDL for 1 qt. jar	MDL for 250ml amber
Nitrogen	0.39		
Methane	0.42	<0.066 mg/l	<0.02 mg/l
Carbon Dioxide	0.58		
Ethylene	0.69		<0.03 mg/l
Ethane	0.78	<0.04 mg/l	<0.03 mg/l
Propane	2.04		

Maintenance:

All maintenance records must be kept in the instrument's maintenance log book. Keep up to date.

Analyte: Methane/Ethane/Ethylene
Matrix: Water

Date Prepared: 4/10/89
By: JMB
Microbac Method:
Revision Date: 5/21/98 BJJ
Approved By:

Quality Control:

1) Run 1% CH_4 reference standard at high sensitivity daily. Do not proceed with analyses unless recovery is $\pm$ 10% of the expected value.
2) Run a duplicate sample every 10 jars or one per batch.
3) Run blank spike and duplicate each day.
4) The analytical range of this procedure is valid from:

   0.02 mg/l - 8 mg/l for CH_4
   0.03 mg/l - 10 mg/l for C_2H_6/C_2H_4

NITRITE

First Environmental Laboratories

Standard Operating Procedure

Title: Nitrite - Diazotization Using Hach Reagent,
Manual Colorimetric Determination

Regulatory References: EPA-600/4-79-020, Method 354.1
Standard Methods, 18th Edition, Method 4500-NO_2 A,B
Note: EPA-600/4-79-020 will not be a valid method reference effective 6/95

Regulatory Limits: NA

Preservation Requirements: No Treatment, Cool 4°C

Container: 250 cc plastic

Single Analysis Sample Volume: 25 mL

Holding Time: 48 hours

(Range) Reporting Limit - Upper End: 0.005 mg/L - 0.250

Summary of Method: Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present.

1. Instrumentation / Apparatus / Glassware

1.1. Spectrophotometer, for use at 543 nm providing a light path of 1 cm.

2. Reagents

All reagents should be prepared using deionized water and volumetric glassware.

2.1. HACH NitraVer 3 Powder Pillow for 25 mL sample volume: Purchased. HACH No. 14065-66 (50 pp / box)

2.2. **SDWA Compliant Procedure** requires the use of the following reagent: add 10 mL 85% phosphoric acid and 1g sulfanilamide to 80 mL DI water. After dissolving sulfanilamide completely, add 0.1g N-(1-naphthyl)-ethylenediamine dihydrogenchloride. Mix to dissolve, then dilute to 100 mL with DI water volumetrically. Expiration Date: 1 month when stored in a dark bottle in refrigerator.

2.3. Concentrated H_2SO_4

3. Standards

3.1. 25 mg/L Nitrite Stock Standard: Dissolve 0.123 sodium nitrite ($NaNO_2$) in DI water and bring to FV of 1L with DI water. Preserve with 1 mL chloroform. Expiration Date: prepare immediately prior to use.

3.2. 0.250 mg/L CCVS: pipet 1 mL of intermediate standard into a 100 mL volumetric and dilute to FV with DI water. Expiration Date: Prepare Fresh Daily

3.3. To Spike the Sample: Pipet 100 uL of stock standard into 25 mL of the sample being spiked. The sample is now spiked with 0.100 mg/L of nitrite.

3.5. Recommended source for ICVS / PE is APG

3.6. Calibration Curve

3.6.1. Purpose: A calibration curve relates instrument response to sample concentration. It also proves that the instrument response, over a determined concentration range, can be predicted using a mathematical equation.

3.6.2. Spectrophotometric analyses require the construction of a calibration curve. **First Environmental Laboratories, Inc.** utilizes a referenced curve system, e.g., a single continuing calibration standard is used to verify the curve on a daily basis.

A standard curve to be referenced requires a blank and five standards evenly distributed throughout the range of the analysis. The data must be collected under the same conditions as those that will exist during routine analyses. Two set of data are generated, each on different days. The results of the calculations generated from each data set are averaged.

3.6.3. Prepare a standard curve by plotting the absorbance values of standards (y-axis) versus the corresponding concentrations (x-axis). Inclusion of the blank in the curve is permitted if the actual instrument response from the blank is used as a data point for constructing the curve. If the spectrophotometer is set to zero with the blank, or if the blank is subtracted out prior to calculating the final result, do not include the blank in the curve. In this instance, including the blank would artificially force the curve through zero.

3.6.4. Frequency: A new calibration curve must be prepared at least yearly. Changes in instrument or reagent conditions, which result in failure to obtain an acceptable response for the CCVS, would indicate the need to prepare a new curve. If more than one analyst performs the analysis, each analyst does not need to have separate curves provided they are able to obtain acceptable results on the CCVS.

3.6.5. Acceptance Criterion: A correlation coefficient ≥ 0.995 must be achieved using all calibration standards. The ICVS must be analyzed immediately following the curve. The result must be within $\pm$ 10% of the true value or within the acceptance ranges established according to the manufacturer of the ICVS. The narrower of the two should normally be applied.

3.6.6. Corrective Action: Since the calibration curve is used for calculating results for all samples and quality control indicators, a curve meeting all requirements must be established prior to analyzing client samples.

Care should be taken when choosing the concentrations of the standards for the calibration curve. If the intercept is large or if the correlation coefficient is poor, then the concentration of the standards used in relation to the detection limit and linear range should be carefully re-evaluated.

Rule of Thumb: Each standard on the curve should back calculate to within $\pm$ 10% of the true value.

3.6.7. Documentation Requirements: The raw data and calculations supporting the use of a referenced curve must be easily retrieved. Record the response factor used to perform the calculations, as well as the reference notation for curve raw data, with the raw data supporting the analysis.

3.6.8. Prepare Calibration Curve: Prepare 0.05, 0.10, 0.15, 0.20, and 0.25 mg/L NO2 standard solution by diluting 0.2, 0.4, 0.6, 0.8, and 1.0 mL of stock solution to 100 mL with deionized water.

4. Analytical Procedures

4.1. Interferences

4.1.1. If an aqueous sample containing particulates, filter the sample through a Whatman No. 40 filter. Alternatively, a background reading may be obtained.

4.1.2. The following ions interfere because of precipitation under test conditions and should be absent: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^{+}, choroplatinate, and metavanadate.

4.1.3. Cupric ion may cause low results by catalyzing decomposition of the diazonium salt.

4.1.4. Very high levels of nitrate (100 mg/L nitrate as N or more) may be slightly reduced to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.

4.2. Sample Preparation

A procedure does not exist for the preparation of a solid matrix sample.

4.3. Determinative Procedure

4.3.1. All samples should be brought to room temperature prior to beginning the analysis. pH adjust samples to between 5 and 9 with 1N HCL or NH_4OH as required.

4.3.2. Set the wavelength to 543 nm on the spectrophotometer.

4.3.3. Measure 25 mL of sample into a disposable culture tube using a graduated cylinder.

4.3.4. If necessary, obtain background readings on acidified sample.

4.3.5. Add the contents of one powder pillow to each tube.

4.3.6. Stopper the tube and invert ten times to dissolve the reagent.

4.3.7. Allow the sample to react for 15 minutes.

4.3.8. Remove the stoppers from the tubes. Zero the instrument on the reagent blank for best results. The blank absorbance should be monitored for trends. Read the absorbance of each tube.

SDWA Compliant Procedure: use 10 mL of sample and 0.4 mL of color reagent in place of the HACH powder pillow. **Note:** A separate curve is required to support the use of this reagent. The sensitivity of the analysis is greater than when using HACH powder pillows.

4.4. Calculation

Include factors resulting from sample preparation and/or the need to analyze the sample at a dilution. The formula provided below assumes the sample was a clean water not requiring dilution.

$$\text{mg/L } NO_2^- \text{ as N} = \text{abs of sample x response factor}$$

where: response factor = $\frac{\text{concentration of standard (mg/L)}}{\text{absorbance of standard}}$ = slope

5. Quality Control

Quality Control Indicator (QCI)	Frequency	Control Limit (Interim)	Control Limit (Statistical)
Calibration Curve	**Referenced**	**cc = 0.995, back calculation of percent recovery with 10% of true value**	**NA**
Initial Calibration Verification	**Immediately following the curve**	**± 10% or within stated limits**	**As supplied by manufacturer**
Reagent Blank / Continuing Calibration Blanks	**Daily at the beginning, end, and after every 10 samples**	**Less than the reporting limit**	**NA**
Continuing Calibration Standard (CCVS) - concentration upper end or middle of range	**Daily at the beginning, end, and after every 10 samples**	**± 10%**	**3 standard deviations for the control limit and 2 standard deviations for the warning limit***
Matrix Spike / Matrix Spike Duplicate (MS/MSD) - concentration 20 to 50 percent of range	**Per batch, where a batch is defined as daily or every 20 samples - whichever is less**	**±25% (SDWA compliant procedures require ±20%)**	**3 standard deviations for the control limit and 2 standard deviation for the warning limit**
*** A control chart is required for either the CCVS or the LCS - not both. The choice is dependent upon whether or not a preparative step is normally utilized, i.e., distillation or digestion.**			

6. Notes

6.1. Tips and Hints

A standardization procedure for verifying the concentration of the stock standard can be found in standard methods. This would only be necessary if the stock solution was retained longer than 12 hours.

6.2. Discussion - Regulatory Compliance

The use of HACH reagents does meet the method requirements and is "approved" according to HACH literature.

6.3. Non-Regulatory References

6.3.1. HACH Water Analysis Handbook, 1989 Hach Chemical Company, page 396-400.

6.3.2. HACH Products for Analysis, 1993-1994.

7. Approvals

Reviewed for Technical Accuracy by: _____

Reviewed for Quality Assurance Compliance by: _____

Implementation Date: _____

End Use Date: _____

ATTACHMENT B

LABORATORY SOPs FOR SOIL ANALYSES

TOTAL ORGANIC CARBON

Determination of Total Organic Carbon in Solid and Semisolid Samples EPA 9060 (Modified)

1.0 Scope and Application

1.1 This method is applicable to the determination of total organic carbon (TOC) in such solid and semisolid samples as river and lake sediments, soil, sludges separated from water and wastewater treatment processes, and sludge cakes from vacuum filtration, centrifugation, or other sludge dewatering processes. Range for this method is 100 to 10,000 mg/kg TOC.

1.2 The sludge and sediment sampler accessory PN 832-222 to the DC-80 Total Carbon Analyzer gives the analyst greater flexibility to measure carbon in a wider variety of sample matrices. This sampler combusts the sample at 850 degrees C in an oxygen atmosphere so that solids as well as liquids can be analyzed. It consists of a magnetically coupled boat inlet system which delivers the sample to the high temperature furnace. Two ports are provided for sample introduction, a septum port for liquid injections, and a flip-top port for solid samples. The boat inlet connects to the analyzer via the PRG-1 module enabling its furnace to serve two functions. Since the high temperature combustion method is used, you can expect higher blanks (approx. 5 ppm C) and devitrification of the combustion tube from samples containing high sodium. To help minimize this problem, we have designed a straight, inexpensive tube which can easily be replaced when necessary. Finally, the electronics control module and detector are all fully compatible with the sampler.

2.0 Sample Collection and Preservation

2.1 100 grams of sample is collected in a glass container with Teflon® lined closure. Keep headspace in the container to a minimum, as this will help prevent the loss of volatile organics. Store samples at 4°C and analyze within 28 days of collection.

3.0 Interferences

3.1 Loss of volatile organic matter during transfer of the sample will cause a negative error.

3.2 There is always a so-called "System Blank". So, for low level TOC analyses, this blank value has to be subtracted.

3.3 The platinum boat may accumulate carbonaceous impurities, mainly from the carrier gas. When the boat is placed at the cool zone for a long period of time, it should be "baked" before use.

4.0 Equipment and Apparatus

4.1 DC-80 Combustion Chamber
- 4.1.1 Combustion Tube: Quartz, 12 mm O.D. X 30 cm
- 4.1.2 Oxidation Promoter/Heat Transfer Agent: Cobalt Oxide on Alumina
- 4.1.3 Sample Boat: Platinum
- 4.1.4 Boat Drive: Manual, magnetically coupled
- 4.1.5 Sample Inlets: Septum port for liquids and suspensions and "Flip-Top" port for solids

4.5 Analytical Balance

4.6 DC-80 TOC Analyzer

5.0 Reagents

5.1 Calibration Standard
- 5.1.1 Dry primary Standard grade Potassium Hydrogen Phthalate (KHP) for a minimum of 2 hours at 105° C. Weigh 0.425 gram into a 100 ml volumetric flask. Dissolve in 10 ml deionized, organic free water. Add 5 drops 1:1 hydrochloric acid and dilute to volume.
- 5.1.2 Standard is 2000 ug/ml carbon.

5.0 Installation and Start-up

5.1 The installation of the solid sampler is easy and straight forward, but the plumbing is slightly different from the PRG Module. Reference the Assembly Drawing, 880-654.

5.2 Referencing the Operating Kit (P/N 899-512) locate all necessary parts to complete the installation.

5.3 Place the PRG Module to the right side of the main DC-80 Reaction Module leaving about 12 inches between. Make sure that power is off.

5.4 Pack 1 ½ to 1 ¾ inch section from the dimple of the combustion tube (P/N 511-533) with oxidation promoter (P/N 511-833) and hold in place with quartz wool.

5.5 Install the glass tube or race (P/N 512-009) on the flip top. Press the race into the clamp at the rear. Loosen four screws of the flip top inlet block and insert the race slowly until it touches the metal stop. Tighten the four screws evenly.

5.6 Remove the bracket from the Flip Top Inlet and attach it to the inlet side of the PRG Module with a screw.

5.7 Attach the Flip Top Inlet to the bracket with two knurled nuts.

5.8 Slide the combustion tube from the exit end through the furnace.

5.9 Loosen the ½" nut at the Flip Top Inlet block and slide the combustion tube in until it touches the metal, then tighten the nut finger tight.

5.10 Place the Boat Carriage (P/N 880-670) in the race, and put the magnets underneath the metal bar of the Carriage. Also place two magnetic Field Extenders (P/N 526-326) over the race.

5.11 Move the magnets so that the boat carrier is in the Flip Top Inlet Block. Open the top and drop the platinum boat (P/N 511-793) in, place a small piece of quartz wool (3-5 mg) into the boat and close the top.

5.12 Connect the gas outlet line from the side panel of the UV Reaction Module (fifth bulkhead from top) to the rear end of the race with 1/8" Teflon tubing and seal with the hole-through rubber stopper, size "000".

5.13 Turn the gas on. **Oxygen must be used.**

5.14 Connect the 1/16" Teflon tubing assembly (P/N 880-666) to the exit of the combustion tube with a rubber stopper. The flared end of tubing should be immersed into a beaker basic water (pH > 10). Make sure that the gas is coming out.

5.15 Turn on the power of the PRG Module and condition the oxidation promoter for 1 hour.

Note 1: For initial 20 minutes or so, NOX will be generated for the combustion tube. Make sure that the end of tubing is immersed in water (pH >10) to absorb NOX.

5.16 After 1 hour, connect the exit Teflon tubing to the side of the UV Reactor Module, at the fourth bulkhead from the top.

5.17 The furnace temperature is preset at the factory at 850° C.

5.18 The sparger and the CO2 scrubber tube of the PRG module are not sued in conjunction with the boat inlet.

6.0 Procedure for Solid Samples

6.1 Sample size: Up to 100 mg. (10-50 mg suggested)

6.2 TOC Range: 100 ppm to 10,000 mg/kg

6.3 Precision (1 standard deviation): ±0.2 ug C. (Assumes no error in weighing sample)

6.4 Analysis time: Typically 5-6 minutes

6.5 In this section, only the operational procedure which is different from the UV-persulfate TOC procedure, will be described. Therefore, a user should be familiar with the operation of the basic DC-80, including the chemical standard preparation. It is important to make sure that when this module is used, the UV lamp is turned off, otherwise poor precision is expected. The pump may also be turned off to conserve reagent.

6.6 In order to inject a sample into the platinum boat, one of two sample inlet ports has to be opened momentarily. This causes a disturbance on the detector baseline. Therefore the timing of when to press the "START" button is important to achieve good results for low level TOC analysis.

6.7 Control Module Setting
- 6.8.1 Mode Selection Switch: TOC
- 6.8.2 Sample Volume Select: 40ul

6.8 Boat Clean Up and System Blank Determination
- 6.8.1 Make sure that the furnace temperature is approximately 850° C (Rather intense red glow is very rough indication of approximately 850° C. Yellowish glow indicates higher temperature).
- 6.8.2 Make sure that:
- 6.8.3 Gas is coming into the UV Reactor
- 6.8.4 Water column height difference in the U-tube is approximately 3-4" (This is typical back pressure indicator).
- 6.8.5 UV lamp is turned off.
- 6.8.6 The reactor is filled with the reagent.
- 6.8.7 A small piece of quartz wool is in the boat. This quartz wool should be replaced daily.

6.9 Observe the detector baseline on the digital display. It should be stable to within $\pm$ 0.0002 for several minutes after the furnace temperature is stabilized and all CO2 is purged out of the reactor.

6.10 Push the boat all the way into the combustion zone and leave it there for 2 minutes.

6.11 Pull back the boat into the Flip Top Inlet Block for 30 seconds to cool off.

6.12 Pull the boat until it is in line with the liquid sample injection port.

6.13 Remove the inlet septum, inject 40ul of deionized of distilled water and put the septum back in.

6.14 Press the "START" right after Step 7. (At least within 5 seconds).

6.15 Slide the boat smoothly into the furnace at about 2 inches per second.

6.16 Standard and Sample Analysis
- 6.16.1 Use a 40 uL injection of the 2000 ug/ml C standard to calibrate the instrument. Analyze as described in paragraphs 6.1 through 6.15.
- 6.16.2 Set the LCD display to read 2000 ppm C.
- 6.16.3 Weigh between 10 and 50 mg of sample into the boat. Combust as described above. Analyze each sample in triplicate.
- 6.16.4 Record the ppm carbon displayed by the LCD.

7.0 Liquid or Slurry Samples

7.1 Follow the same procedure as outlined above.

7.2 When using the flip top inlet for solid samples, you must allow 2 minutes after closing the top for baseline to return to normal. Then push the start button and slide the sample into the furnace.

8.0 Calculations

8.1 Caculate the mg/kg carbon in the sample by the following equation:

mg/kg Carbon = 40 / A * B

Where: A = Sample amount, in mg
B = ppm Carbon reading from LCD display

9.0 Quality Assurance/Qualtiy Control

9.1 Analyze each sample in triplicate. Average the results for reporting. Individual replicates should agree within 20%.

9.2 A duplicate sample is analyzed every 10 samples. Duplicate analyses should agree within 20%.

9.3 A method blank is analyzed with each batch (i.e. 20 samples or less). Use low sodium sand which has been baked at 600° C to remove organic matter. The blank should be below the reportable detection limit.

9.4 Each day samples are analyzed also analyze an independent commercial reference standard. Use ERA Standard #542, TOC in Soil, or equivalent. Results should agree within 10% of the reference value.

10.0 Data Validation

10.1 Individual triplicates of the same sample should agree within 20%

10.2 Duplicate sample analysis should agree within 20%.

10.3 Method blank should be non-detectable.

10.4 Daily external reference standard should be within 10% of the stated value.

Health and Safety

Use appropriate safety procedures. Samples are non-preserved and should not pose a hazard. However, safe laboratory practices should be followed.

References

SW-846 Method 9060.

DC-80 Instrument Manual.

NITRATE

Analysis of Nitrate, Nitrogen by use of an Ion Selective Electrode SM 4500-NO_3^- D

1.0 Scope and Application

1.1 This method is applicable to the measurement of nitrate in drinking, surface and saline waters, domestic and industrial wastewater, and soil and sludges.

1.2 The nitrate ion electrode is a selective sensor that develops a potential across a thin, porous, inert membrane that in place a water-immiscible liquid ion exchanger. The electrode responds to nitrate ion activity between about 0.14 to 1400 mg/L nitrate-nitrogen. The lower limit of detection is determined by the small but finite solubility of the liquid ion exchanger.

1.3 The range of this method for Nitrate-Nitrogen is 1 - 1000 mg/L for water samples and 10 -10,000 mg/kg for soil/sludge.

2.0 Sample Preservation

2.1 Water Samples

2.1.1 Collect 100 mL of sample in glass or plastic container. Sample should be stored at 4° C and preserved with 1:1 Sulfuric Acid to pH <2. Analyze preserved samples within 28 days of collection.

2.2 Soil/sludge Samples

2.2.1 Collect 100 grams of sample in glass or plastic container. Sample should be stored at 4° C. Analyze within 28 days of collection.

3.0 Interferences

3.1 Chloride and bicarbonate ions interfere when their weight ratios to NO_3^--N are >10 or >5, respectively.

3.2 Ions such as nitrite, cyanide, sulfate, bromate, etc. may interfere but are normally not at significant concentrations in potable waters.

4.0 Equipment and Apparatus

4.1 pH/ISE Meter, Orion Model 710A

4.2 Double junction reference electrode, Orion Model 90-20

4.3 Nitrate ion electrode, Orion Model 93-07

4.4 Magnetic stirrer and Teflon coated stir bars

5.0 Reagents

5.1 Buffer solution: Dissolve 34.64 g aluminum sulfate octadecahydrate, 6.86 g silver sulfate, .2.56 g boric acid, and 5.04 g sulfamic acid in 1500 mL of reagent water. Adjust to pH 3.0 by slowly adding 0.1 N NaOH. Dilute to 2 L and store in a dark glass bottle.

5.2 Reference electrode filling solutions
- 5.2.1 Inner Chamber: Orion Cat. No. 900002
- 5.2.2 Outer chamber: Dissolve 0.53 g ammonium sulfate in 100 mL of reagent water or dilute 2 mL of Orion Cat. No. 930711 Ionic Strength Adjuster to 100 mL.

5.3 Stock nitrate solution: Dissolve 0.7218 g KNO_3 in reagent water and dilute to 100 mL. Preserve with 0.2 mL chloroform. NO_3-N conc. = 1000 mg/L.

5.4 Dilute 10 mL of Solution 5.3 to 100 mL. NO_3-N = 100 mg/L.

5.5 Nitrate check standard: Dissolve 0.607 g $NaNO_3$ in reagent water and dilute to 100 mL.

5.6 Nitrate free reagent water.

5.7 Nitrate Extraction fluid. Dissolve 5.28 grams of ammonium sulfate in appx. 500 mLs of reagent water and add 31 mLs of 2% boric acid. Dilute to 1 liter.

6.0 Procedure

6.1 Electrode Preparation and Meter Set-up
- 6.1.1 Nitrate Electrode
  - 6.1.1.1 Screw sensing module into the electrode body, making sure rubber washer is in place, until finger-tight.
- 6.1.2 Reference Electrode
  - 6.1.2.1 Unscrew electrode cap, sliding cap and spring up the cable.
  - 6.1.2.2 Push down on top of inner chamber until cone at bottom of electrode can be grasped using a tissue.
  - 6.1.2.3 Remove inner chamber from electrode body and slide rubber sleeve down to expose filling hole.
  - 6.1.2.4 Fill inner chamber with Solution 5.2.1. Shake chamber down like a thermometer if having trouble filling chamber.
  - 6.1.2.5 Replace inner chamber and screw cap on until finger-tight. (Do not overtighten.)
  - 6.1.2.6 Add a small amount of Solution 5.2.2 to outer chamber.
  - 6.1.2.7 Tip it to wet O-ring on electrode body and return to upright position.
  - 6.1.2.8 Hold electrode by cap and push up on the barrel of the electrode until some filling solution wets the inner cone. Release the electrode body and inner cone should return to normal position.
  - 6.1.2.9 Fill outer chamber with Solution 5.2.2. *Note: See Electrode Instruction Manuals for maintenance and storage information.*

6.1.2.10 Attach electrodes to meter and adjust mode to concentration.

6.2 Calibration

6.2.1 Prepare the following series of standards in 50 mL beakers:

Std	mL Reagent Water	mL Soln. 5.4	mg/L
1	9.9	0.10	1.0
2	9.75	0.25	2.5
3	9.5	0.50	5.0
4	9.0	1.0	10.0
5	0.0	10.0	100.0

6.2.2 Add 10 mL of buffer soln. (4.1) and a stir bar to each beaker

6.2.3 Press "2nd" key. Press "cal" key. P1 will be displayed. Lower electrodes into std 1. Wait until meter indicates "ready". Press "timer" key. The decimal point will begin to flash. Press "timer" key until decimal point is in the second position from left. Press "yes" key. The first number on left will begin to flash. Use up or down arrow keys to adjust display to 1. Press "yes" key. The next number will begin flashing. Use arrow keys to adjust to "0". Press "yes" key. Continue these steps until the fourth digit is entered. P2 will then be displayed.

6.2.4 Rinse electrodes and lower into std. 2. Repeat the procedure in 6.2.3 for all standards entering the appropriate concentration.

6.2.5 After the fifth standard is entered a slope will momentarily be displayed. Record this value.

6.2.6 Before proceeding with analysis run a 10 mg/L check standard prepared by adding 0.1 mL of soln. 5.5 to 9.9 mLs reagent water and 10 mL buffer soln. This must be within ± 10% of 10.

6.2.7 Also run an LCS in the range of 1 to10 mg/L for an additional verification of the curve. This must be within ± 10% of the "true" value.

6.3 Samples - water

6.3.1 Add 10 mL of sample to 10 mL of buffer. Rinse electrodes and lower into sample. Record the concentration when meter indicates ready.

6.3.2 If sample concentration exceeds upper calibration std. make an appropriate dilution and reanalyze..

6.4 Samples - soils/sludges

6.4.1 Weigh approximately 5 g of sample into a 150 mL beaker and add 50 ml of nitrate extraction fluid.

6.4.2 Agitate vigorously on magnetic stirrer for 10 minutes.

6.4.3 Allow a few minutes for particulates to settle.

6.4.4 Follow steps 6.3.1 through 6.3.2 using the supernatant. Use the extraction fluid in place of reagent water in preparation of the standards.

7.0 Calculations

7.1 Calculate NO_3-N concentration of soil/sludge as follows:

$$NO_3\text{-N, mg/kg, dry weight} = \frac{A \times B}{C \times D}$$

Where: A = NO_3-N conc, mg/L
B = L of reagent water used in 6.4.1
C = kg of sample weighed in 6.4.1
D = % solids of sample

8.0 Quality Assurance/Quality Control

8.1 Spike of minimum of one sample in every twenty.
8.2 Run a duplicate analysis of one sample in every twenty.
8.3 Run a check standard or LCS after every ten samples and after the last sample.

9.0 Data Validation

9.1 Initial 10 mg/L check standard must be within 10% of the true value. If not a new curve must be prepared.
9.2 If check standard deviates more than ± 10%, recalibrate and repeat analysis of all samples analyzed since last good check std.
9.3 Spike sample must be with ± 15%.
9.4 Duplicate or duplicate spike samples must be within 20% relative percent difference. If recoveries are outside these limits contact supervisor.

10.0 Health and Safety

10.1 Appropriate protective equipment should be worn when handling samples and chemicals.
10.2 Read appropriate MSDS information on chemical safety.

11.0 References

11.1 *Standard Methods for the Examination of Water and Wastewater,* 18th Ed., Method 4500-NO_3 D, p.4-88.

Table 1

mL of Stock Standard	Conc. of Stock Std. mg/L	mL of Reagent Water	Conc. of Calib. Std., mg/L
1	10	9	1.0
2	10	8	2.0

3	10	7	3.0
4	10	6	4.0
5	10	5	5.0
7	10	3	7.0
10	10	0	10.0
0.5	1000	9.5	10
1.0	1000	9.0	50.0
5.0	1000	5.0	500
10	1000	0	1000

SULFATE

Analysis of Sulfur as Sulfate in Soils by Inductively Coupled Plasma EPA 6010

1.0 Scope and Application

1.1 This method is used for the determination of sulfur as sulfate in soils.

1.2 An acid digestion procedure is employed to prepare the samples for analysis.

1.3 Method Summary

1.3.1 The atomic emission of samples are measured by an optical spectroscopic technique. Samples are nebulized and the aerosol that is produced is transported to the plasma torch where excitation occurs. Characteristic atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

2.0 Sample Preservation

2.1 Samples should be kept at 4 C.

3.0 Interferences

3.1 Laboratory glassware and equipment can cause contamination.

3.2 Impure reagents

3.3 Spectral interferences

3.4 High dissolved solids in samples

3.5 High acid concentrations in samples

3.6 Sample matrix interferences

3.7 For additional information on interferences see Reference 11.1.

4.0 Equipment and Apparatus

4.1 Inductively Coupled Plasma, Atomic Emission Spectrometer, Leeman Labs, PS1000, Computer Controlled

4.2 Argon gas supply

5.0 Reagents

5.1 Sulfate stock standard, 3000 mg/L. Dissolve .513 grams of hydroxyl amine sulfate in appx. 75 mLs of reagent water and bring to volume in a 100 mL volumetric flask.

5.2 Sulfate calibration standard, 300mg/L. Dilute 10 mL of soln. 5.1 to 100 mL.

5.3 Sulfate check standard, 100 mg/L. Dilute 3.33 mL of soln. 5.1 to 100 mL.

5.4 All acids used for standard preparation are of a quality suitable for trace element analysis.

5.3 Reagent water, Type II, is used for standard preparation.

6.0 Procedure

6.1 Sample preparation

6.1.1 Weigh 1-2 g (dry weight) of a representative sample to the nearest 0.01 g directly into a beaker.

6.1.2 Add 10 mL of 1:1 nitric acid, mix to a slurry and cover with a watch glass. Heat to 95° C and reflux for 15 minutes without boiling. Cool.

6.1.3 Add 5 mL of concentrated nitric acid, replace watch glass and reflux for 30 minutes. Repeat this step a second time.

6.1.4 Remove watch glass and evaporate to approximately 5 mL. Cool beaker.

6.1.5 Add 2 mL of reagent water and 3 mL of peroxide. Replace watch glass and heat gently until effervescence subsides. Cool beaker.

6.1.6 Continue adding peroxide in 3 mL aliquots with warming until effervescence is minimal or sample appearance is unchanged. Do Not add more than 10 mL of peroxide.

6.1.7 Transfer to 100 mL graduated cylinder.. Rinse beaker 3 or 4 times and transfer to cylinder. Dilute to 100 mL with reagent water. Allow solids to settle or filter before analyzing.

6.2 Instrument Set-up

6.2.1 Power - 1 kw

6.2.2 Coolant Flow - 14 LPM

6.2.3 Nebulizer Pressure - 35-45 PSI

6.2.4 Auxiliary Flow - .3 LPM

6.2.5 Pump Rate - 1.5 mL/min.

6.2.6 Autostart Coolant - 14 LPM

6.2.7 Instrument should be allowed to operate at least 15 minutes after torch is ignited before beginning any analysis.

6.2.8 Prior to calibrating instrument a peak optics and peak source routine are run.

6.3 Calibration

6.3.1 Open a protocol containing sulfur as an analyte.

6.3.2 Open a folder to store all data which is generated.

6.3.3 The instrument is calibrated daily by running a calibration blank and a 300 mg/L standard.

6.3.4 Linearity of calibration curve is demonstrated by running 100 mg/L standard.

6.3.5 Check standards must be within ± 15% of "true" values before proceeding with analysis.

6.3.6 An independent laboratory control standard is run to demonstrate the accuracy of the calibration curve.

6.4 Samples

6.3.1 Uptake time is 45 seconds to allow sufficient time for sample to reach plasma and stabilize.

6.3.2 Between samples the system is rinsed with calibration blank for 60 seconds.

6.3.3 If sample concentration exceeds the highest calibration standard, the instrument can be recalibrated using a higher concentration standard or an appropriate dilution of the sample can be analyzed.

7.0 Calculations

7.1 If dilutions are performed, use appropriate dilution factor to determine sample value.

7.2 Samples should be reported in mg/kg up to three significant figures:

$$SO4,\ mg/kg = \frac{\text{Conc., mg/L x 0.1 L x dilution factor}}{\text{kg sample x \% solids}}$$

8.0 Quality Assurance/Quality Control

8.1 A check standard is analyzed at a frequency of 10%.

8.2 A reagent blank is brought through the complete analytical process for each batch of samples.

8.3 A spike and spike duplicate sample are analyzed at a frequency of 5%.

9.0 Data Validation

9.1 If check standard does not agree within 15% of true value, the instrument is recalibrated and all samples analyzed subsequent to the previous check standard are reanalyzed.

9.2 Results of the reagent blank analysis must not exceed the MDL.

9.3 Spike recovery should be 70-130%. If not within this range, contact supervisor.

9.4 Duplicate samples must have a relative percent difference within 20%. If outside of this range, contact supervisor.

10.0 Health and Safety

10.1 Use appropriate safety procedures. For specific information of reagents used in this procedure consult the MSDS sheets.

11.0 Reference

11.1 *Test Methods for Evaluating Solid Waste, SW 846.*

(See next page for Table 1)

Table 1

Element	Wavelength, nm	PS 1000 Identifier
Aluminum	396.152	Al 2
Antimony	206.833	Sb 1
Arsenic	193.695	As 2
Barium	455.403	Ba 1
Beryllium	313.042	Be 1
Cadmium	214.438	Cd 1
Calcium	317.933	Ca 3
Chromium	267.720	Cr 4
Copper	324.754	Cu 1
Iron	259.940	Fe 2
Lead	220.353	Pb 1
Magnesium	285.213	Mg 3
Manganese	257.610	Mn 1
Molybdenum	202.030	Mo 1
Nickel	231.604	Ni 3
Phosphorus	213.618	P 1
Potassium	766.490	K 1
Selenium	196.026	Se 1
Silver	328.068	Ag 1
Sodium	589.592	Na 2
Sulfur	180.669	S 1
Thallium	190.801	Tl 1
Vanadium	292.401	V 3
Zinc	213.856	Zn 1

pH

METHOD 9045C

SOIL AND WASTE pH

1.0 SCOPE AND APPLICATION

1.1 Method 9045 is an electrometric procedure for measuring pH in soils and waste samples. Wastes may be solids, sludges, or non-aqueous liquids. If water is present, it must constitute less than 20% of the total volume of the sample.

2.0 SUMMARY OF METHOD

2.1 The sample is mixed with reagent water, and the pH of the resulting aqueous solution is measured.

3.0 INTERFERENCES

3.1 Samples with very low or very high pH may give incorrect readings on the meter. For samples with a true pH of >10, the measured pH may be incorrectly low. This error can be minimized by using a low-sodium-error electrode. Strong acid solutions, with a true pH of <1, may give incorrectly high pH measurements.

3.2 Temperature fluctuations will cause measurement errors.

3.3 Errors will occur when the electrodes become coated. If an electrode becomes coated with an oily material that will not rinse free, the electrode can (1) be cleaned with an ultrasonic bath, or (2) be washed with detergent, rinsed several times with water, placed in 1:10 HCl so that the lower third of the electrode is submerged, and then thoroughly rinsed with water, or (3) be cleaned per the manufacturer's instructions.

4.0 APPARATUS AND MATERIALS

4.1 pH Meter with means for temperature compensation.

4.2 Glass Electrode.

4.3 Reference electrode: A silver-silver chloride or other reference electrode of constant potential may be used.

> NOTE: Combination electrodes incorporating both measuring and referenced functions are convenient to use and are available with solid, gel-type filling materials that require minimal maintenance.

4.4 Beaker: 50-mL.

4.5 Thermometer and/or temperature sensor for automatic compensation.

4.6 Analytical balance: capable of weighing 0.1 g.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Reagent water. All references to water in this method refer to reagent water, as defined in Chapter One.

5.3 Primary standard buffer salts are available from the National Institute of Standards and Technology (NIST) and should be used in situations where extreme accuracy is necessary. Preparation of reference solutions from these salts requires some special precautions and handling, such as low-conductivity dilution water, drying ovens, and carbon-dioxide-free purge gas. These solutions should be replaced at least once each month.

5.4 Secondary standard buffers may be prepared from NIST salts or purchased as solutions from commercial vendors. These commercially available solutions, which have been validated by comparison with NIST standards, are recommended for routine use.

6.0 SAMPLE PRESERVATION AND HANDLING

6.1 All samples must be collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 Samples should be analyzed as soon as possible.

7.0 PROCEDURE

7.1 Calibration:

7.1.1 Because of the wide variety of pH meters and accessories, detailed operating procedures cannot be incorporated into this method. Each analyst must be acquainted with the operation of each system and familiar with all instrument functions. Special attention to care of the electrodes is recommended.

7.1.2 Each instrument/electrode system must be calibrated at a minimum of two points that bracket the expected pH of the samples and are approximately three pH units or more apart. Repeat adjustments on successive portions of the two buffer solutions until readings are within 0.05 pH units of the buffer solution value. If an accurate pH reading based on the conventional pH scale [0 to 14 at 25°C] is required, the analyst should control sample temperature at 25±1°C when

sample pH approaches the alkaline end of the scale (e.g., a pH of 11 or above).

7.2 Sample preparation and pH measurement of soils:

7.2.1 To 20 g of soil in a 50-mL beaker, add 20 mL of reagent water, cover, and continuously stir the suspension for 5 minutes. . Additional dilutions are allowed if working with hygroscopic soils and salts or other problematic matrices.

7.2.2 Let the soil suspension stand for about 1 hour to allow most of the suspended clay to settle out from the suspension or filter or centrifuge off the aqueous phase for pH measurement.

7.2.3 Adjust the electrodes in the clamps of the electrode holder so that, upon lowering the electrodes into the beaker, the glass electrode will be immersed just deep enough into the clear supernatant solution to establish a good electrical contact through the ground-glass joint or the fiber-capillary hole. Insert the electrodes into the sample solution in this manner. For combination electrodes, immerse just below the suspension.

7.2.4 If the sample temperature differs by more than 2°C from the buffer solution, the measured pH values must be corrected.

7.2.5 Report the results as "soil pH measured in water at __ °C" where "__°C" is the temperature at which the test was conducted.

7.3 Sample preparation and pH measurement of waste materials:

7.3.1 To 20 g of waste sample in a 50-mL beaker, add 20 mL of reagent water, cover, and continuously stir the suspension for 5 minutes. . Additional dilutions are allowed if working with hygroscopic wastes and salts or other problematic matrices.

7.3.2 Let the waste suspension stand for about 15 minutes to allow most of the suspended waste to settle out from the suspension or filter or centrifuge off aqueous phase for pH measurement.

NOTE: If the waste is hygroscopic and absorbs all the reagent water, begin the experiment again using 20 g of waste and 40 mL of reagent water.

NOTE: If the supernatant is multiphasic, decant the oily phase and measure the pH of the aqueous phase. The electrode may need to be cleaned (Step 3.3) if it becomes coated with an oily material.

7.3.3 Adjust the electrodes in the clamps of the electrode holder so that, upon lowering the electrodes into the beaker, the glass electrode will be immersed just deep enough into the clear supernatant to establish good electrical contact through the ground-glass joint or the fiber-capillary hole. Insert the electrode into the sample solution

in this manner. For combination electrodes, immerse just below the suspension.

7.3.4 If the sample temperature differs by more than 2°C from the buffer solution, the measured pH values must be corrected.

7.3.5 Report the results as "waste pH measured in water at __ °C" where "__°C" is the temperature at which the test was conducted.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for the appropriate QC protocols.

8.2 Electrodes must be thoroughly rinsed between samples.

9.0 METHOD PERFORMANCE

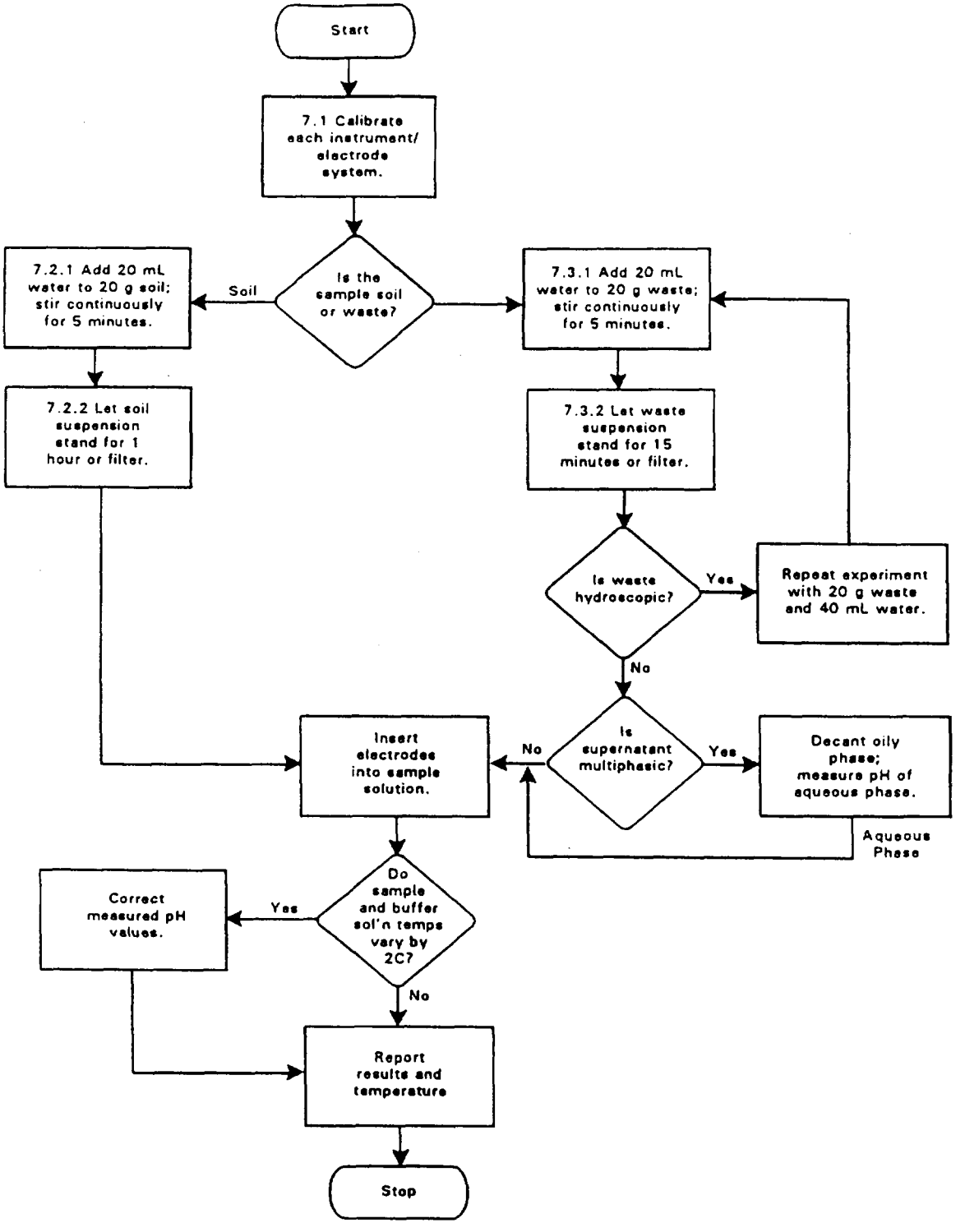
9.1 No data provided.

10.0 REFERENCES

1. Black, Charles Allen; Methods of Soil Analysis; American Society of Agronomy: Madison, WI, 1973.

2. National Bureau of Standards, Standard Reference Material Catalog, 1986-87, Special Publication 260.

SOIL AND WASTE pH



TOTAL SOLIDS

Determination of Total Solids in Solid and Semisolid Samples EPA 160.3

1.0 Scope and Application

1.1 This method is applicable to the determination of total solids in such solid and semisolid samples as river and lake sediments, sludges separated from water and wastewater treatment processes, and sludge cakes from vacuum filtration, centrifugation, or other sludge dewatering processes.

2.0 Sample Preservation

2.1 200 grams of sample is collected. Sample should be analyzed as soon as possible. Samples must be analyzed within 7 days. Store samples at 4°C.

3.0 Interferences

3.1 Loss of ammonium carbonate and volatile organic matter during drying will cause a negative error.

3.2 Weighings must be done quickly as wet samples tend to lose weight by evaporation and dried samples are often hygroscopic and rapidly absorb moisture from the air.

4.0 Equipment and Apparatus

4.1 Metal weigh boats
4.2 Dessicator
4.3 Analytical balance, capable of weighing to 1.0 mg
4.4 Drying oven, 103 to 105°C

5.0 Reagents

5.1 Not applicable

6.0 Procedure

6.1 Total Solids

6.1.1 Heat weigh boat at 103 to 105°C for 1 hour.

6.1.2 Cool to room temperature in a dessicator and weigh to nearest 1 mg..

6.1.3 Store weigh boat in dessicator until ready to use.

6.1.4 Fluid sample analysis

6.1.4.1 If sample contains enough moisture to flow more or less readily, stir to homogenize, place 25 to 50 grams in a prepared weigh boat, and weigh.

6.1.4.2 Dry at 103 to 105°C overnight.

6.1.4.3 Cool sample in dessicator until at room temperature, and weigh.

6.1.4.4 Repeat heating, cooling, desiccating, and weighing procedure until the weight change is less than 4% or 50 mg, whichever is less.

6.1.5 Solid sample analysis

6.1.5.1 Pulverize the entire sample coarsely on a clean surface by hand, using rubber gloves.

6.1.5.2 Put 25 to 50 grams in prepared weigh boat and weigh.

6.1.5.3 Dry in an oven at 103 to 105°C overnight.

6.1.5.4 Cool to room temperature in desiccator and weigh.

6.1.5.5 Repeat drying, cooling, weighing, and desiccating steps until weight change is less than 4% or 50 mg, whichever is less.

7.0 Calculations

7.1 Total Solids

$$\% \text{ total solids} = \frac{(A-B) \times 100}{C-B}$$

Where: A = weight of residue and dish in mg
B = weight of dish
C = weight of wet sample + dish, mg

8.0 Quality Assurance/Qualtiy Control

8.1 A duplicate sample is run every 10 samples

8.2 Samples should be weighed until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 50 mg, whichever is less.

9.0 Data Validation

9.1 Duplicate samples should agree within 5%.

10.0 Health and Safety

10.1 Use appropriate safety procedures. Samples are non-preserved and should not pose a hazard. However, safe laboratory practices should be followed.

11.0 References

11.1 *Method 2540G, Standard Methods for the Examination of Water and Wastewater.*

11.2 *ESEPA Method 160.3*

TOTAL HETEROTROPHS

Enumeration of Heterotrophic Bacteria for Bioremediation Biofeasability

1.0 Scope and Application

1.1 This method is applicable to the enumeration of heterotrophic bacteria in soils or waters for the purpose of evaluating the biofeasability of in situ bioremediation.

1.2 Summary of Method

1.2.1 Enumeration of bacteria grown on a nonselective media.

2.0 Sample Preservation

2.1 Sample requires a 500 ml plastic nonpreserved bottle.

2.2 Sample must be analyzed within 5 days.

3.0 Interference's

3.1 Samples should not be preserved or frozen as this could kill bacteria in the sample.

4.0 Equipment and Apparatus

4.1 Automatic pipettor, range 0.1 to 1.0 ml

4.2 Sterile pipettor tips, range 0.1 to 1.0 ml

4.3 Automatic pipettor, range 0.01 to 0.2 ml

4.4 Sterile pipettor tips, range 0.01 to 0.2 ml

4.5 Propane torch

4.6 Potters wheel

4.7 Glass rod (bent into a hockey stick shape for spreading)

4.8 Incubator (Plexiglas airtight enclosure)

4.9 Sterile petrie dishes

4.10 Sterile 10 ml serological pipettes

4.11 20 ml glass test tubes

4.12 Mellenkamp colony counter.

5.0 Reagents

5.1 Sterile phosphate buffer solution (PBS): Dissolve 2.34g of Na_2HPO_4-$7H_2O$, 0.18g NaH_2PO_4 and 8.5g NaCl in 1L of reagent water. After all has dissolved dispense 95 ml into 150 ml milk dilution bottles and autoclave at 121 degrees C for 30 to 35 minutes. After autoclaving, let them cool and check the volume of one bottle using a 100 ml graduated cylinder, should be 90 ml + or - 2 ml.. Also check the sterility of one bottle by removing 40 ml of buffer solution and adding 50 ml of double strength tryptic soy broth and incubating it for 24 hours. If the sterility check is

turbid after incubation or if the volume is out of range the whole batch must be discarded. Store bottles away from light

5.2 Nutrient Broth (NB): Dissolve 4g of dehydrated nutrient broth into 1L of reagent water. Dispense into tubes, 10 ml each. Cap and autoclave for 15 minutes at 121 degrees celsius. After the tubes have been autoclaved, check the sterility of one by incubating if at room temperature for 24 hours. If, after incubation, the broth is turbid the whole batch must be discarded. Also take another tube and inoculate it with some contaminant degrading bacteria and incubate for 24 hours. If this tube does not produce turbidity within 24 hours then the whole batch must be discarded. Store tubes in the cooler.

5.3 Nutrient Agar (NA): Dissolve 4g of dehydrated nutrient broth and 15g of agarose into 1L of reagent water. Cap and autoclave at 15 minutes and at 121 degrees. After autoclaving, put the solution on a stir plate to stir until it is cool enough to hold in your hand. Once it can be held, pour the agar into sterile petrie dishes. Pour just enough to cover the bottom of the petrie dishes. Keep the plates upright until they have cooled and solidified. Once they have solidified, store plates inverted away from light. Be sure to discard any plates that have growth on them.

5.4 Ethanol, laboratory grade.

6.0 Procedure

6.1 Preparation

6.1.1 Take the samples out of the cooler and allow them to warm up to room temperature.

6.1.2 Obtain a copy of the chain of custody and locate the site name. In the bioremediation logbook, find the last time samples from this site were analyzed.

6.1.3 The samples should be set on the same media as they were the last time that site was analyzed. For example, if the last time that site was analyzed they were set in tubes then the new samples from that site should also be set on tubes. If the samples are to be set in tubes then the tubes should be removed from the cooler and allowed to warm up to room temperature.

6.1.4 If no samples from this site had been analyzed previously, then Check the chain of custody or the quote to find out what media to use. If neither of these specifies what media to use, then use what media had been used for previous samples from the company that sent the samples.

6.1.5 Determine the appropriate dilution's to set by finding the results from the last time samples from the same site were set. The dilution's to be set may have to be adjusted higher or lower based on the previous results. If the site had not been previously set then set 10^{-2} through 10^{-6}.

6.1.6 Record the sample numbers, site name, matrix (soil or water), date set, initials of the analyst, media on which the samples will be set, and the dilution's that will be set in the bioremediation logbook.

6.1.7 **All samples must be set in duplicate**. This includes both duplicate dilutions and duplicate tubes or plates.

6.1.8 Prepare a series of PBS bottles labeled with the sample number and the appropriate dilution factors, 10^{-1} though one factor less than the highest dilution to be set. For example, if the dilution's to be set are 10^{-2} though 10^{-6} then a series of PBS bottles are to be prepared labeled 10^{-1} through 10^{-5}.

6.1.9 Prepare the 10^{-1} dilution.

6.1.9.1 For waters transfer 10 ml of the sample, using a sterile pipette, into the PBS bottle labeled 10^{-1}.

6.1.9.2 For soils transfer 10 g of the sample, using a sterile spatula, (sterilize spatula by rinsing it in bleach then rinsing thoroughly in reagent water) into the PBS bottle labeled 10^{-1}.

6.1.10 Prepare the rest of the dilution's by transferring 10 ml of the 10^{-1} dilution into the PBS bottle labeled 10^{-2}. Repeat this procedure with the 10^{-2} dilution and the PBS bottle labeled 10^{-3} and so on until all dilution's contain the appropriate amount of sample.

6.1.11 If a count of contaminant degrading bacteria is also required the same dilution bottles may be used for them as well.

6.2 NB tubes

6.2.1 Using an automatic pipettor with a sterile tip, pipette 0.1 ml of the first dilution into each of five NB tubes.

6.2.2 Label the first of the five tubes previously inoculated one factor more than the dilution. For example, the tubes inoculated from the 10^{-2} dilution should be labeled 10^{-3}.

6.2.3 Repeat steps 6.2.1 and 6.2.2 until all the dilution's have been set into NB. The dilution's to be set were determined in step 6.1.5.

6.2.4 Incubate at room temperature for 5 to 7 days.

6.2.5 After incubation the tubes must be read to see if they are positive or negative. A positive tube is one that is turbid. A turbid tube is any tube that is more cloudy than the sterility blank.

6.26 Deterimine if each tube is positive or negative and record the result with a + or - in the appropriate place in the logbook.

6.2.7 Calculate the MPN/ml for the sample.

6.2.8 For the final result, average the results of the sample and its duplicate and report as MPN/ml.

6.3 BIMA plates

6.3.1 For every dilution to be set label 2 NA plates. Be sure to label the plates on the bottom.

6.3.2 Pipette 0.1 ml of the first dilution onto the NA plates labeled one factor higher than the PBS dilution bottle. For example, the PBS

dilution 10^{-2} should be plated on the NA plates labeled 10^{-3}. Also, after the NA plates have been inoculated be sure to keep them upright.

6.3.3 Sterilize the bent glass rod by dipping it into ethanol and using the propane torch to light the ethanol on the glass rod. While doing this, be sure to hold the glass rod at a down angle so the burning ethanol does not flow down to your hand.

6.3.4 After the ethanol has burned off and the glass rod has cooled, place one NA plate on the potters wheel and give it a spin. While that NA plate is spinning use the sterilized glass rod to spread the 0.1 ml of sample dilution evenly across the NA plate and put the lid back on the plate. Repeat this step for the other NA plate.

6.3.5 Sterilize the glass rod again.

6.3.6 Repeat steps 6.3.2 through 6.3.5 for the rest of the dilution's that were determined to be set in step 6.1.5.

6.3.7 Incubate the NA plates inverted for 5 to 7 days.

6.3.8 After incubation, use the colony counter to count the colonies on each plate that was set and record the counts in the appropriate space in the logbook. A good way to keep track of which colonies have been counted and which have not is to use a marker when counting the colonies.

6.3.9 Also record the characteristics of the colonies growing on the plates in the comments section of the bioremediation book as well. Characteristics to be recorded include, but are not limited to, smooth or varigated, opaque or translucent, and the color of the colony.

6.3.10 Calculate the colony forming units per milliliter, (CFU/ml).

6.3.11 For the final result, average of the results of the sample and its duplicate and report as CFU/ml.

7.0 Calculations

7.1 MPN/ml =

$$\frac{\text{total no. of positive tubes x 10,000}}{(\text{ml sample in negative tubes x ml sample in all tubes})^{1/2}}$$

7.2 CFU/ml =

$$\frac{\text{total no. of colonies on all plates}}{\text{total volume (in ml) of sample plated}}$$

8.0 Quality Assurance and Quality Control

8.1 NB tubes

8.1.1 Positive and negative controls must be set for every site. The positive control must be positive and the negative control must be negative or the data must be flagged

8.1.1.1 Sterility Blank (negative control): one NB tube with nothing added.

8.1.1.4 Positive control: one NB tube with some contaminant degrading bacteria added.

8.2 BIMA plates

8.1.2 One plate for each sample must be inoculated with sterile PBS dilution water and incubated and counted with the plates for that sample. The control must have less than 30 colonies or the data must be flagged.

9.0 Health and Safety

9.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined, therefore all should be treated as potential health hazards. Gloves, eye protection, and a lab coat should be worn when handling reagents for this procedure.

AEROBIC HYDROCARBON DEGRADERS

Enumeration of Bacteria Capable of Degrading a Designated Contaminant

1.0 Scope and Application

1.1 This method is applicable to the enumeration of bacteria in soils or waters able to degrade a designated contaminant for the purpose of evaluating the biofeasability of in situ bioremediation.

1.2 Summary of Method

1.2.1 Enumeration of bacteria grown on media that provides only the contaminant as a nutrient.

2.0 Sample Preservation

2.1 Sample requires a 500 ml plastic nonpreserved bottle.

2.2 Sample must be analyzed within 5 days.

3.0 Interference's

3.1 Samples should not be preserved or frozen as this could kill bacteria in the sample.

4.0 Equipment and Apparatus

4.1 Automatic pipettor, range 0.1 to 1.0 ml

4.2 Sterile pipettor tips, range 0.1 to 1.0 ml

4.3 Automatic pipettor, range 0.01 to 0.2 ml

4.4 Sterile pipettor tips, range 0.01 to 0.2 ml

4.5 Propane torch

4.6 Potters wheel

4.7 Glass rod (bent into a hockey stick shape for spreading)

4.8 Incubator (Plexiglas airtight enclosure)

4.9 Sterile petrie dishes

4.10 Sterile 10 ml serological pipettes

4.11 20 ml glass test tubes

4.12 Mellenkamp colony counter.

5.0 Reagents

5.1 Sterile phosphate buffer solution (PBS): Dissolve 2.34g of Na_2HPO_4-$7H_2O$, 0.18g NaH_2PO_4 and 8.5g NaCl in 1L of reagent water. After all has dissolved dispense 95 ml into 150 ml milk dilution bottles and autoclave at 121 degrees C for 30 to 35 minutes. After autoclaving, let them cool and check the volume of one bottle using a 100 ml graduated cylinder, should be 90 ml + or - 2 ml.. Also check the sterility of one bottle by removing 40 ml of buffer solution and adding 50 ml of double strength tryptic soy broth and incubating it for 24 hours. If the sterility check is

turbid after incubation or if the volume is out of range the whole batch must be discarded. Store bottles away from light.

5.2 BIMB tubes: Dissolve 0.80 g K_2HPO_4, 0.20g KH_2PO_4, 0.05 $CaSO_4$-$2H_2O$, 0.50 $MgSO_4$-$7H_2O$, 0.01 $FeSO_4$-$7H_2O$, 1.00g $(NH_4)_2SO_4$, and 0.075 ml of a 1% resazurin solution into 1L. After all this has dissolved, dispense 10 ml into culture tubes. Cap the culture tubes and autoclave at 121 degrees C for 15 minutes. After the tubes have cooled inoculate one tube with gas degrading bacteria and add 0.010 ml of a 10 ppm gasoline solution and incubate that tube with one that has not been inoculated for 48 hours. The tube that was inoculated should change color after incubation and the other one should still be blue, if this is not the case then the batch must be discarded. Store the tubes in the cooler.

5.3 BIMA plates: Dissolve 0.80 g K_2HPO_4, 0.20g KH_2PO_4, 0.05 $CaSO_4$-$2H_2O$, 0.50 $MgSO_4$-$7H_2O$, 0.01 $FeSO_4$-$7H_2O$, 1.00g $(NH_4)_2SO_4$, and 20g of agarose into 1L in an autoclavable container containing a magnetic stirbar. Autoclave the solution for 20 minutes at 121 degrees C. After autoclaving remove the solution from the autoclave, be careful solution will be very hot, and place it on a magnetic stir plate to cool. When the solution has cooled to the point where it can be held comfortably pour the solution into sterile petrie dishes, pour only enough agar to cover the bottom of the petrie dishes. After the petrie dishes containing agar have cooled and solidified, store them inverted away from light at room temperature.

5.4 Designated contaminant: A 10 ppm solution of the designated contaminant is required for BIMB tubes and a pure form is required for BIMA plates.

5.5 Ethanol, laboratory grade.

6.0 Procedure

6.1 Preparation

6.1.1 Take the samples out of the cooler and allow them to warm up to room temperature.

6.1.2 Obtain a copy of the chain of custody and locate the site name. In the bioremediation logbook, find the last time samples from this site were analyzed.

6.1.3 The samples should be set on the same media as they were the last time that site was analyzed. For example, if the last time that site was analyzed the degraders were set in tubes then the new samples from that site should also be set on tubes. If the samples are to be set in tubes then the tubes should be removed from the cooler and allowed to warm up to room temperature.

6.1.4 If no samples from this site had been analyzed previously, then Check the chain of custody or the quote to find out what media to use. If neither of these specifies what media to use, then use what

media had been used for previous samples from the company that sent the samples.

6.1.5 Determine the appropriate dilution's to set by finding the results from the last time samples from the same site were set. The dilution's to be set may have to be adjusted higher or lower based on the previous results. If the site had not been previously set then set 10^{-2} through 10^{-6}.

6.1.6 Record the sample numbers, site name, matrix (soil or water), date set, initials of the analyst, media on which the samples will be set, and the dilution's that will be set in the bioremediation logbook.

6.1.7 **All samples must be set in duplicate**. This includes both duplicate dilutions and duplicate tubes or plates.

6.1.8 Prepare a series of PBS bottles labeled with the sample number and the appropriate dilution factors, 10^{-1} though one factor less than the highest dilution to be set. For example, if the dilution's to be set are 10^{-2} though 10^{-6} then a series of PBS bottles are to be prepared labeled 10^{-1} through 10^{-5}.

6.1.9 Prepare the 10^{-1} dilution.

6.1.9.1 For waters transfer 10 ml of the sample, using a sterile pipette, into the PBS bottle labeled 10^{-1}.

6.1.9.2 For soils transfer 10 g of the sample, using a sterile spatula, (sterilize spatula by rinsing it in bleach then rinsing thoroughly in reagent water) into the PBS bottle labeled 10^{-1}.

6.1.10 Prepare the rest of the dilution's by transferring 10 ml of the 10^{-1} dilution into the PBS bottle labeled 10^{-2}. Repeat this procedure with the 10^{-2} dilution and the PBS bottle labeled 10^{-3} and so on until all dilution's contain the appropriate amount of sample.

6.1.11 If a count of heterotrophic bacteria is also required the same dilution bottles may be used for them as well.

6.2 BIMB tubes

6.2.1 Using an automatic pipettor with a sterile tip, pipette 0.1 ml of the first dilution into each of five BIMB tubes.

6.2.2 Label the first of the five tubes previously inoculated one factor more than the dilution. For example, the tubes inoculated from the 10^{-2} dilution should be labeled 10^{-3}.

6.2.3 Using an automatic pipettor with a sterile tip, pipette 0.01 ml of the 10 ppm designated contaminant solution into each of the five tubes previously inoculated.

6.2.4 Repeat steps 6.2.1 through 6.2.3 until all the dilution's have been set into BIMB. The dilution's to be set were determined in step 6.1.5.

6.2.5 Incubate at room temperature for 5 to 7 days.

6.2.6 After incubation the tubes must be read to see if they are positive or negative. The rezazurin in the BIMB is a sky blue color unless

it has been metabolized, when it turns pink. A positive is considered any tube that is pink or clearly lavender. The best way to determine if a lavender tube is positive or negative is to compare it to the negative control. If the tube is not noticably different from the control then it is negative.

6.2.7 Deterimine if each tube is positive or negative and record the result with a + or - in the appropriate place in the logbook.

6.2.8 Calculate the MPN/ml for the sample.

6.2.9 For the final result, average the results of the sample and its duplicate and report as MPN/ml.

6.3 BIMA plates

6.3.1 For every dilution to be set label 2 BIMA plates. Be sure to label the plates on the bottom.

6.3.2 Pipette 0.1 ml of the first dilution onto the BIMA plates labeled one factor higher than the PBS dilution bottle. For example, the PBS dilution 10^{-2} should be plated on the BIMA plates labeled 10^{-3}. Also, after the BIMA plates have been inoculated be sure to keep them upright.

6.3.3 To sterilize the bent glass rod, dip it into ethanol and use the propane torch to light the ethanol on the glass rod. While doing this, be sure to hold the glass rod at a down angle so the burning ethanol does not flow down to your hand.

6.3.4 After the ethanol has burned off and the glass rod has cooled, place one BIMA plate on the potters wheel and give it a spin. While that BIMA plate is spinning use the sterilized glass rod to spread the 0.1 ml of sample dilution evenly across the BIMA plate and put the lid back on the plate. Repeat this step for the other BIMA plate.

6.3.5 Sterilize the glass rod again.

6.3.6 Repeat steps 6.3.2 through 6.3.5 for the rest of the dilution's that were determined to be set in step 6.1.5.

6.3.7 Incubate the BIMA plates inverted in the presence of contaminant fumes for 5 to 7 days.

6.3.8 After incubation, use the colony counter to count the colonies on each plate that was set and record the counts in the appropriate space in the logbook. A good way to keep track of which colonies have been counted and which have not is to use a marker when counting the colonies.

6.3.9 Also record the characteristics of the colonies growing on the plates in the comments section of the bioremediation book as well. Characteristics to be recorded include, but are not limited to, smooth or varigated, opaque or translucent, and the color of the colony.

6.3.10 Calculate the colony forming units per milliliter, (CFU/ml).

6.3.11 For the final result, average of the results of the sample and its duplicate and report as CFU/ml.

7.0 Calculations

7.1 MPN/ml =

$$\frac{\text{total no. of positive tubes x 10,000}}{(\text{ml sample in negative tubes x ml sample in all tubes})^{1/2}}$$

7.2 CFU/ml =

$$\frac{\text{total no. of colonies on all plates}}{\text{total volume (in ml) of sample plated}}$$

8.0 Quality Assurance and Quality Control

8.1 BIMB tubes

8.1.1 Positive and negative controls must be set for every site. The positive control must be positive and the negative controls must be negative or the data must be flagged

8.1.1.1 Sterility Blank (negative control): one BIMB tube with nothing added.

8.1.1.2 Methanol Blank (negative control): one BIMB tube with 0.01 ml of methanol added.

8.1.1.3 Contaminant Blank (negative control): one BIMB tube with 0.01 ml of the contaminant solution added.

8.1.1.4 Positive control: one BIMB tube with 0.01 ml of the contaminant solution and some contaminant degrading bacteria added.

8.2 BIMA plates

8.1.2 One plate per sample must be inoculated with sterile PBS dilution water and incubated and counted with the plates for that sample. The controls must have less than 30 colonies or the data must be flagged.

9.0 Health and Safety

9.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined, therefore all should be treated as potential health hazards. Gloves, eye protection, and a lab coat should be worn when handling reagents for this procedure.

ACRIDINE ORANGE COUNTS

Microbe Inotech Labs, Inc.

Standard Operating Procedure Number

MIC-011-1

Title: Acridine Orange Direct Count for Total Microbial Count

Reviewed by: Kirk Hartwein 7/16/97 (sign/date)
Kirk Hartwein
Lab Manager

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Supersedes *SOP #: Not Applicable Date: Not Applicable*

Exact copy of original.
LMB 7/17/97
Montgomery-Watson 6/12/98

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1.0 Purpose & Overview

The purpose of this SOP is to outline the methodology [9216 B. Epifluorescence Microscopic Method] for direct total cell counts of bacteria in water, wastewater, groundwaters and soils. Such counts usually exceed counts obtained from heterotrophic plate counts and most probable number methods because, unlike those procedures, direct counts preclude errors caused by viability-related phenomena such as selectivity of growth media, cell clumping, and slow growth rates.

2.0 Definitions

SOP = Standard Operating Procedure

3.0 Scope

The epifluorescence microscopic method produces direct total cell counts with relative speed and sensitivity. It does not permit differentiation of bacterial cells on the basis of taxonomy, metabolic activity, or viability, and it cannot be used to estimate the microbial biomass because of considerable variation in the volume of individual cells. The method requires an experienced technician [Senior Staff or Lab Manager] who can distinguish microbial cells in all their varieties from debris on the basis of morphology. The method consists of sample fixation for storage, staining with a chemical fluorochrome [acridine orange], vacuum filtration onto a nonfluorescing polycarbonate membrane, and enumeration by counting with an epifluorescence microscope

4.0 Materials

a. *Microscope*, vertical UV illuminator for epifluorescence with flat field 100 x oil immersion objective lens, to give total magnification of at least 1000x.

b. *Counting graticule*, ocular lens micrometer calibrated with a stage micrometer.

c. *Filters*, including:
excitation filters (Zeiss KP490 and LP455),
beam splitter (LP510), and
barrier filter (IP520 using mercury lamp, HBO 50)
similar filters from the microscope vendor are acceptable

d, *Vortex mixer*.
e. *Filtration unit*, suitable for use with 25mm dia. membrane filters.
f. *Membrane filters*, polycarbonate, 25mm dia., 0.2µm pore size
g. *Syringes*, 3 mL, disposable, with disposable syringe filter, 0.2 µm pore size.
h. *Test tubes*, glass, screw-capped, 13 x 125mm.

Reagents

a. *Phosphate buffer*:
Dissolve 13.6 g reagent grade KH_2PO_4 in deionized, distilled water and dilute to 1 L. Adjust to pH 7.2, if necessary, by dropwise addition of reagent grade NaOH or HCl. Filter through 0.2µm membrane filter.

b. *Fixative*, 5% (w/v) glutaraldehyde in phosphate buffer. Prepare fresh daily.
c. *Fluorochrome*, 0.01% (w/v) acridine orange in phosphate buffer.
d. *Immersion oil*, low fluorescing.

5.0 Procedure

5.1 Preparation

For collected water samples:

- Set up a 10-fold dilution series for total plate counts and document in the raw data. Clean water samples may not require dilution, but a larger sample volume (>100 mL) may be required to obtain reliable counts.
- Sample for viable plate counts, if desired.
- Add 1.0 mL fixative and document in the raw data. Fixed samples can be stored at 4°C for up to 3 weeks without significant decrease in cell numbers.

For samples from mesotrophic or eutrophic sources or soils disperse and dilute samples as follows:

- Mix sample using a vortex mixer on high setting for 10 minutes.
- Set up a 10-fold dilution series in phosphate buffer, as necessary (typically 10^{-1}, 10^{-3} and 10^{-5} dilutions). Document in the raw data.
- Place 1mL sample or dilution on a nonfluorescent polycarbonate filter supported in a filter holder.
- Using disposable sterile syringe filters, add 1 mL acridine orange reagent solution and wait 2 minutes. Add approximately 3 mL filtered phosphate buffer to promote more even cell distribution. **Alternatively, combine fluorochrome with sample in a small clean vial, let react, and add mixture to filter holder.**
- Filter with vacuum (ca. 13 kPa).
- Wash with 2 mL phosphate buffer and filter.
- Remove polycarbonate filter with forceps and air dry for 1 to 2 min. The filter can be cut into quarter sections and saved if needed.
- Place dried filter on a small drop of immersion oil on a clean glass microscope slide.
- Add a small drop of immersion oil to filter surface.
- Gently cover filter with a clean glass cover slip. Samples can be stored in the dark for several months without significant loss of fluorescence; however, we typically do not do this unless specifically requested.

5.2 Plate Count

- Examine at least 10 randomly selected fields on the filter using the 100x oil immersion lens to establish that distribution of microbial cells is uniform and that individual cells can be enumerated. The optimum dilution series will derive 10-50 cells per field. If dilution was not sufficient, go back to the appropriate preparation section of the SOP and repeat at a higher dilution series.
- When optimum dilution has been obtained, count the number of cells in 100 squares using the calibrated counting graticule (this is the typical situation). If cell growth is greater than optimum, count 20 squares. If cell growth is less than optimum, count more than 100 squares. Record the number of cells counted and the number of squares counted in the raw data.

5.3 Calculations

Calculate the average number of cells per filter. Obtain effective filter area from specifications of filtration unit. Show all calculations in the raw data. The following formula is used to determine the number of cells per milliliter of sample:

Total cells/mL = (avg cells/square) x (squares/filter) x (dilution factor)/ sample volume, mL.

6.0 Revising the SOP

7.0 References

9216 B. Epifluorescence Microscopic Method. pp.9-39 and 9-40. *Standard Methods For the Examination of Water and Wastewater*, 18th Edition 1992.

Hobbie, J.E., F.J. Daley & S. Jasper. 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33:1225.

Sierack, M.E., P.W. Johnson & J. McH. Sieburth. 1985. Detection, enumeration, and sizing of planktonic bacteria by image-analyzed epifluorescence microsopy. *Appl. Environ. Microbiol.* 49:799.

American Society for Testing and Materials. 1987. Standard test method for enumeration of aquatic bacteria by epifluorescence microscopy counting procedure. ASTM D4455-85, Annual Book of ASTM Standards, Vol. 11.02, Water. American Soc. Testing & Materials, Philadelphia, Pa.

21 CFR Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies, July 31, 1991.

21 CFR Part 210 Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or holding of Drugs.

40 CFR Part 160 Federal Insecticide, Fungicide and Rodenticide Act; Good Laboratory Practice Standards, August 17, 1989.

40 CFR 792 Toxic Substances Control Act; Good Laboratory Practice Standards, August 17, 1989.

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